A Summary of Current Program 7/1/66

and Preliminary Report of Progress

for 7/1/65 to 6/30/66

ENTOMOLOGY RESEARCH DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

Section B

This progress report is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1965, and June 30, 1966. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D.C.

July 1, 1966

TABLE OF CONTENTS

AREA NO. 13. LIVESTOCK INSECTS AND OTHER ARTHROPODS

(Includes previous areas 13, 14, 15, & 16)

Problem. Insects and other related arthropods attack all classes of livestock and poultry causing estimated annual losses of $877,850,000. Losses are attributed to direct attack of arthropods causing losses in weight gains and milk and egg production and losses in the value of livestock products such as meat, hides, and wool. Additional losses result from livestock and poultry diseases spread by arthropod vectors. A large variety of insects and other arthropods affect livestock including mosquitoes and biting gnats, house flies, horse flies and deer flies, ticks and keds, the face fly, the stable fly, the horn fly, cattle grubs and other bots, lice, mites, and fleeceworms. Practical but not adequate control methods for many of these livestock pests have been developed, but satisfactory methods of protecting livestock and poultry from mosquitoes, biting gnats, horse flies, deer flies, and stable flies remain an unsolved problem. Development by insects of resistance to control chemicals is a continuing potential threat to current effective methods of control employing chemicals. The occurrence of insecticide residues in meat and animal products restricts the usefulness of some chemical control methods. Continued basic and applied research is needed to develop new, safer, more effective chemical control agents and methods of using them as well as other methods, such as management practices, sterilization, attractants, and biological control into highly effective integrated means of control or eradication. Research is also needed to study the role of insects in the spread of diseases of livestock and poultry.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing, long-term program involving basic and applied research on the biology and control of insects and related arthropods which affect the health and productivity of all classes of livestock. The total Federal scientific effort devoted to research on livestock insects is 37.8 scientific man-years and includes studies on: (1) beef, horse and swine insects; (2) dairy cattle insects; (3) sheep and goat insects, and (4) poultry insects. Research is conducted on: (A) basic biology, physiology, and nutrition; (B) conventional insecticide control methods; (C) insect parasites, predators, and pathogens; (D) insect sterility and other new approaches to control; (E) attractants and repellents; (F) insect vectors of diseases; and (G) program leadership. The following tabulation indicates the distribution of scientific man-years between commodity groups (1-4) and types of research (A-G).

	A	B	C	D	E	F	G	Totals
1	6.2	2.2	1.8	3.3	2.4	0.4	0.6	16.9
2	4.6	2.2	1.0	3.3	1.7	0.4	1.0	14.2
3	1.2	0.8	0.3	1.0	0.3	1.0	0.2	4.8
4	---	1.2	---	0.5	---	---	0.2	1.9
Totals	12.0	6.4	3.1	8.1	4.4	1.8	2.0	37.8

Federal support in research grants, contracts, and extended cooperative agreements provides for 11.8 scientific man-years per 3 years. Commodity distribtuion is 4.8 to beef insects; 3.6, to dairy insects; 2.2, to poultry insects and 1.1, to sheep and goat insects. Research area distribution is 5.1 to basic biology, physiology, and nutrition; 1.6, to conventional insecticide control methods; 3.0, to insect parasites, predators, and pathogens; 0.8, to insect sterility and other new approaches to control; and 1.3 to attractants and repellents. Grants, contracts, and extended cooperative agreements are located at the following institutions: University of California at Berkeley, University of California at Davis, University of Georgia, Athens, University of Kentucky, Lexington, McNeese State College, Lake Charles, La., University of Southwestern Louisiana, Lafayette, Louisiana State University and A&M College, Baton Rouge, Mississippi Agricultural Experiment Station, State College, New Mexico State University, Las Cruces, University of Utah, Salt Lake City, Virginia Polytechnic Institute, Blacksburg, University of Wyoming, Mississippi State University, and Instituto Nacional de Investigaciones Agricolas, Secretaria de Agricultura y Ganaderia Estados Unidos Mexicanos.

Additional research is conducted under grants supported by P. L. 480 funds. A13-ENT-3, entitled, "Investigations on the biology of dung beetles in Korea and their role in the prevention of fly breeding in dung", is under study at the Department of Agricultural Biology, College of Agriculture, Seoul National University, Suwon, Korea; A10-ENT-12, entitled "Laboratory study of tick repellents and acaricides", at the Veterinary Institute, Beit Dagan, Israel, and S9-ENT-6 entitled, "Investigations on natural enemies of ants" at Ministerio de Ganaderia y Agricultura, Montevideo, Uruguay.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 36.5 professional man-years is devoted to this area of research.

PROGRESS USDA AND COOPERATIVE PROGRAM

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes (All livestock). At Gainesville, Fla., bacterial infusions of hay and fresh grass have been found to stimulate growth in Aedes aegypti, Anopheles quadrimaculatus, Aedes triseriatus, Aedes taeniorhynchus, and Culex quinquefasciatus. In preliminary tests these infusions have also been found to stimulate oviposition and egg hatch and induce autogeny. There is also some indication that they contain substances which attract mosquitoes. Research is now under way to determine whether bacteria or chemical substances are responsible for these stimulations and responses.

Tests conducted to determine whether females of Culex p. quinquefasciatus mated more than once showed that females did not re-mate and one mating was sufficient to satisfy mating requirements of females.

At Lake Charles, La., studies on the biology of mosquitoes were continued. The blood feeding and oviposition behavior of Aedes canadensis was studied. One blood meal was required to produce one batch of eggs. In the laboratory, females would not take more than 3 blood meals or lay more than 3 egg batches. None of the eggs hatched showing that this species is univoltine in this area. In an attempt to compare the degree of hatching of eggs of some floodwater species of Aedes with the number of times the eggs were flooded with water, open and screened enclosures were build over breeding sites on marsh areas. In one of four sets of open and screened enclosures, it appeared that all of the hatchable eggs had been depleted after numerous floodings over one year. The remaining screened enclosures, continued to produce sizeable but smaller broods of larvae than the open enclosures.

At Corvallis, Oreg., studies were continued on the basic biology of mosquitoes. Exposure of Culex quinquefasciatus females to low levels of light during the night influenced their behavior and response to attractants the following day. Surveys on mosquito abundance indicated that C. tarsalis and C. peus were less abundant in the Willamette Valley in 1965 than in previous years. During the fall of the past 3 years larvae of Aedes increpitus have been collected rather widely in the flood plain of the Willamette River indicating that this increpitus strain is well-adapted to the region.

Research has been continued under two contracts at the University of Southwestern Louisiana and McNeese State College. Light trap collections have shown the relative abundance and dispersion of different species of mosquitoes. Correlations of the occurrence of floodings and high tides were made and showed that the numbers of Aedes sollicitans trapped during the second quarter of 1965 were higher than the numbers of Culex salinarius and this trend was reversed during the 3rd and 4th quarter. Data on temperature, rainfall, and the direction and velocity of wind are being gathered to correlate with abundance and dispersal of various species. The seasonal activity of several important species of mosquitoes was

determined in two areas in Louisiana. Aedes sollicitans and C. salinarius were present and active throughout the year except for short periods of no significant rain or high tides. Culiseta inornata was caught only in the cooler months; while Mansonia perturbans and Psoropha confinnis were active only in the warm months. Anopheles quadrimaculatus was absent from one area and relatively scarce in the other. Culex quinquefasciatus was found only in one spot near a residential area where a few premises were not well cared for.

2. House Flies (All livestock). At Corvallis, Oreg., research was continued on the basic biology of the little house fly. Field collected, p^{32} labeled little house flies were released for migration studies. Flies dispersed throughout a poultry farm and fur farm within 24 hours. Studies were conducted on the mating behavior of the little house fly. Males were shown to be capable of successfully fertilizing at least 7 females each. Preliminary tests indicate females may mate more than one time. Wild-collected females deposited eggs that were 72 to 82% viable. Excess numbers of males caged with females did not increase female mortality or decrease fecundity or fertility.

3. Horse Flies and Deer Flies (All livestock except poultry). At Stoneville, Miss., eggs were obtained from 16 species of Tabanidae in a specially developed oviposition chamber. Tabanus proximus, T. vittiger schwardti and T. atratus oviposited more readily in confinement than other species. The eggs of several species had never before been observed. The egg masses of each of the species is distinctive, thus providing a means of identification of the species in the absence of adults. These findings together with the development of techniques for rearing the larvae in the laboratory represent significant advancements in our knowledge of the Tabanidae. The incubation for most of the species has been determined and the larvae of some species have been reared to maturity. Several species are fairly easily reared and one, T. vittiger schwardti has been successfully colonized. The larval period of this species ranged from 104 to 147 days in spring rearings to only 50 to 63 days in the fall. The pupal period ranged from 4 to 11 days. Differences in developmental rates presumably are related to temperature. In some groups of larvae 39% have survived to pupate and 72% of these have produced adults. The life cycle of other species varied greatly. The larval period of T. abdominalis ranged from 212 to 526 days; T. proximus from 522 to 547 days; T. lineola from 107 to 195 days. Pupal periods from these three species were 14-15, 12 and 4-9 days, respectively.

At Stoneville, Mississippi, a 12-day study (July 12-23) was conducted to determine the number and species of tabanids attracted to bait animals and the percentage of these that actually fed. Landing rates were highest from 8 to 10 AM and 2 to 4 PM, whereas, the percentage feeding were highest from 10 AM to 2 PM when populations were low to moderate. There was some correlation between percent feeding and solar

radiation, but an inverse correlation between solar radiation and landing rates. The effects of temperature and humidity were not clearly evident from data obtained and further studies will be necessary to determine their influence on adult Tabanid behavior. Feeding rates for the various species of tabanids ranged from 3.2% for T. lineola and 6.5% for T. vittiger schwardti (the most numerous species) to 100% for T. proximus, with the overall average being 30%.

Special studies were conducted in Mississippi, to elucidate the feeding habits of several important species of horse flies. It was found that several species were capable of taking 2 blood meals and T. atratus took 3. These species oviposited after both the 1st and 2nd blood meals.

At Kerrville, Texas, extremely large populations of horse fly larvae were found in fecal matter in a dairy barn drainage area. About 80% of the larvae were T. atratus and 20% T. vittiger schwardti. The larvae of these species are predaceous. Apparently they were feeding mainly on the abundant supply of other dipterous larvae.

Research has been conducted under grant support at the University of Wyoming on the biology of tabanids. Specimens were collected throughout the state for identification of species, densities of populations, and time of activity. Females of Hybomitra frontalis were maintained in the laboratory for 19 days on a diet of sugar, dry Dimalt and water. Observations indicated tabanids in Wyoming tended to land on the head, shoulders, and sides of cattle. They preferred darker areas of cattle. Motion influenced attraction of the tabanids.

4. Screw-worm (All livestock except poultry). A Mexican strain of screw-worm flies has been derived from 62 individual egg masses collected in Veracruz, Mexico and 9 combined collections of egg masses from Sinaloa, Mexico. All cross matings were controlled to insure the maximum genetic variability in the final gene pool. This strain is being tested for possible use in Mexico by the Southwestern Screw-worm Eradication Program.

After 7 generations of laboratory colonization, Mexican strain screw-worms still have a longer developmental cycle on artificial medium than those of the Florida strain. Larvae of the latter strain leave the medium after 70 to 77.5 hr as compared to about 85 hr for larvae of the Mexican strain.

Mating frequency among male screw-worm flies appears to be a function of the degree of adaptation to laboratory conditions. Mean matings per male in one hour increased from 1.5 to 7.5 after a newly introduced strain of screw-worms had been reared through 18 generations in the laboratory. Sexual aggressiveness tests on males from 6 strains of flies reared in the laboratory for 5, 15, 26, 41, 69, and 100

generations also indicated that harassment of females by males increased with time in culture. Apparently both size and strain affect mating activity. Large males flies are more successful than small males of the same strain in mating with large female flies. This difference is greater when females from a recently colonized strain are used.

Studies were continued on the rate of development of screw-worm larvae in artificial media and in wounds on animals. Larvae in horsemeat medium reached the 3rd instar in 36 to 48 hours after hatching while a period of 70 to 78 hours was required by larvae in fresh wounds on sheep. More than 90% of the larvae had left the horsemeat medium after 96 hours. Larvae in fresh wounds on sheep required 168 hours to complete feeding. Larvae placed in previously infested wounds on sheep developed as rapidly as those on artificial medium, but remained on the animals 24 hours longer in the 3rd instar.

A field test was run for 6 months near Poza Rica, Veracruz, Mexico to compare the efficacies of 2 diets used for rearing the sterilized screw-worm flies being released in the Southwest Screwworm Eradication Program. Wounded test animals, arrayed systematically over 2 separate but similar test areas, were used to attract oviposition by native female screw-worms. One test area was treated with sterile flies raised on horsemeat, the other area with flies raised on the liquid diet. Both areas received 420 flies per square mile per week, dropped by airplane along fixed flight lanes 12 miles apart. Ratios of sterile to fertile egg masses laid on the wounded test animals were used to measure relative efficacy of the diets. Sterilized flies raised on horsemeat induced slightly more egg mass sterility than did those raised on liquid diet, but the differences were not striking, and the ratios of sterile to fertile egg masses ran lower than expected throughout the test. Sterile egg masses appeared more often directly under the flight lanes than either 3 or 6 miles away, but again the differences were not great.

A 4-month comparison of burros with sheep for use as wounded test animials in attracting oviposition by native female screw-worm flies in Mexico demonstrated that the cost of preventing the burros from ridding themselves of screw-worm eggs or larvae was too great to make them a practical substitute for sheep.

An analysis of the population dynamics of screw-worms in Mexico was accomplished by employing a method for making contour maps of isopleths of reported screw-worm infestations. Three major areas of infestation exist year-around with apparent independence of each other, one on the Caribbean coast below 20° N Lat., a second on the Pacific coast below 19° N Lat. and the third on the Pacific coast between 19° and 26° N Lat. All other parts of Mexico experience marked annual declines (or losses) of screw-worm populations due to severe cold and/or drouth and seem to derive new populations from the 3 major areas. Some

overwintering does occur outside the major year-around centers of activity. The analysis provides quantitative estimates of case abundance, by seasons, and suggests ways to relate case abundance to numbers of sterile flies needed for eradication and/or control.

Studies were continued on the reproductive behavior of the screw-worm fly. Few matings with virgin 5-day-old flies were recorded for each sex at 1 day of age, but the number of successful matings rose rapidly to 3 days of age. Thereafter, male sexual aggressiveness declined and female receptivity increased very slightly to 14 days of age. Almost 75% of copulating 1-week-old males transferred sperm within 15 seconds of commencement of mating. A maximum of about 90% of males transferred sperm when copulations approached completion.

Newly emerged females exposed to sexually mature males for 1-10 days began laying eggs of normal viability on the 3rd day and oviposition increased to the 5th day. When newly emerged males were exposed to sexually mature females for 1-10 days, oviposition and hatchability were low after 1 day of exposure, but oviposition was maximal for the 2nd day and hatchability was maximal for the 3rd day.

Two groups of 5-day-old mated females were subjected to 0.5±1.5°C for one hour allowed to recover at 27°C, and 24 hours later the females of 1 treated and an untreated group were each combined with untreated males in a 1:2 ratio. The cold treatment had no adverse effect on survival or egg production. A reduction in hatchability of eggs from mature mated females treated with cold was attributed to inactivation of some of the sperm stored in the females. Elevated hatchability of eggs from mature mated females treated with cold and offered an opportunity to remate suggested that some partially deseminated females mated again.

Females isolated individually for the first 5 days of adult life and then mated to 5-day-old males were less fecund and fertile than females maintained together in groups of 50 prior to mating.

The length of unselected puparia were measured with a metric micrometer caliper and later associated with the sex of the contained fly upon its emergence. Male puparia were longer than female puparia within a single rearing group, but there were differences in length between one rearing group and another.

5. Stable Fly (Cattle and horses). At Kerrville, Texas, studies showed that male and female stable flies normally remain in copulation from 3 to 7 minutes. From 60 to 90 percent of the females were inseminated in the first mating. Results indicated that female flies probably do no remate if sperm is transferred in the first mating.

In Texas, studies were undertaken to study the responses of stable

flies to photoperiods. Pupae within two days of emergence of adults were used in all tests. Results showed that females produced an average of 77 eggs under continuous light, 56 eggs under continous darkness and only 16 eggs under 12 hours light and 12 hours darkness.

At Corvallis, Oregon, studies were undertaken on the feeding habits and nutrition of stable flies. Studies have shown that blood feeding is required by females for the production of viable eggs; however, males can successfully fertilize females without feeding on blood. Both sexes survived well on carbohydrate diets and/or sources of protein other than blood but no viable eggs were produced. Attempts are being made to select strains with differing nutrient requirements for egg production.

6. Face Fly (Cattle and horses). At Corvallis, Oregon, surveys were made for the presence of face flies, Musca autumnalis. This fly has not been reported in Oregon, but has recently been discovered in two neighboring States, Idaho and Washington. Two surveys in Central, Eastern, and Northeastern Oregon failed to show the presence of face flies.

7. Cattle Grubs. At Kerrville, Texas, studies were continued to obtain more information on the biochemical and physiological requirements of second and third instar larvae of cattle grubs and to develop methods for rearing larvae under laboratory conditions. A satisfactory synthetic media was developed but bacterial contamination made it impossible to keep larvae alive over 2 weeks. This problem was partially overcome by sterilizing the larvae in a saline - antibiotic wash and transferring them to sterile containers containing culture media. Subsequently, an automatic system was developed for feeding the larvae in cells in sponges. This system avoided the use of excess media and larval survival increased markedly.

In Texas, several improved synthetic media were developed for studies on rearing cattle grubs under laboratory conditions. The rate of increase in weight of cattle grub larvae in these media was twice that in those previously tried. Past problems of contamination were largely overcome by sterilizing cattle gullets prior to removal of grub larvae and by keeping the larvae in sterile media. In subsequent tests ultraviolet light further improved bacterial control. In this environment some first instar larvae survived for over 90 days but none molted to the second instar. Recent studies have shown that first stage grub larvae survive longer when maintained in small cheese cloth bags. Survival and development has also been enhanced by the use of an improved automatic feeding device.

In preliminary studies improved emergence of flies from pupae was obtained by varying holding temperatures from 5° to 25° C rather than

holding at a constant temperature.

In Texas, in 1965, H. lineatum larvae first appeared in the backs of cattle early in October, and reached a peak December 10 - January 7, with 80% being in the third stage. By February 18, practically all were of this stage, whereas the first few larvae of H. bovis were just appearing.

In Oregon, studies were conducted to observe the behavior, pupation site preference and duration of the prepupal stage of H. bovis cattle grub larvae newly emerged from the backs of cattle. The larvae tended to burrow under anything in their paths, such as plants, roots, sticks and cattle droppings, but others preferred to pupate at the bases of clumps of grass or other vegetation. The larvae appeared to be negatively phototropic regardless of weather conditions. Migration times ranged from 6.0 to 77.0 hours and distance traveled ranged from 6.5 to 110 inches. Pupation sites were covered with cages and observed regularly for adult emergence. The pupation period ranged from 30 to 39 days for larvae pupating from May 5-12 and from 40 to 49 days for those pupating April 20 - May 2. Nearly 80% of the pupae produced adults. Mean dates of adult emergence were June 11-16. Average adult longevity ranged from 3.9 to 5.2 days. In the laboratory the duration of the pupal stage averaged only 20 days and the adults lived about a day longer than field emerged adults.

8. Horn Fly (Cattle). In Texas, techniques were perfected for mass rearing of horn flies under laboratory conditions. The flies were confined in 8.5 inch square plastic screen cages under continuous light. Absorbent cotton pads soaked with beef blood were provided for food. The two sexes copulate readily. Females drop their eggs at random and they fall through the bottom screen cages onto trays. The eggs are then counted and placed in trays of manure. Under these conditions the average time from egg to adult is 12 days and from egg to egg, 17 days. A colony of 50-60,000 flies is being maintained for experimental uses.

An artificial medium was developed in order to produce a uniform colony which was not attainable because of the wide variation in cow manure, the normal rearing medium. The media consists of 22 g of sugar cane pulp, 4 g of whole wheat flour, 500 mg of sodium bicarbonate, 300 mg of dehydrated ox gall and 110 ml of water. This medium was the best of a large number tried and produced uniform flies for resistance tests and other experimental uses.

Studies were continued in Texas, on the selection of horn flies for resistance to ronnel. The colony showed about 100-fold resistance after 43 generations of selection. An even higher degree of resistance was evident after 48 generations, with topical applications of 1.0 ug/fly causing only 38% mortality. Efforts to induce resistance to

toxaphene were unsuccessful.

9. Ticks (Cattle). At Kerrville, Texas, a 5-year study on the seasonal abundance of ticks on cattle was completed. The lone star tick (Amblyomma americanum (L.)), the most abundant species, was found on cattle throughout the year; some in February, rapidly increasing in numbers until activity peaked in March-June, rapidly decreasing in numbers through August, with only a few ticks present from September to January. Peak populations of adult lone star ticks, as determined by dragging pastures, coincided with peaks of infestations on cattle. The winter tick (Dermacentor albipictus (Pochard)), another abundant species, appeared first on cattle in October; activity peaked in November - January and decreased rapidly in February-March until almost no ticks were found in April-September. The black-legged tick (Ixodes scapularis Say), a less common species, appeared on cattle in October; activity peaked in November-December, few ticks were found in April-September. Ears of animals were examined and the abundance of nymphal ear ticks (Otobius megnini (Duges)) were recorded. This species had an inconsistant peak in late summer,-early fall, but the seasonal relationship was not well defined.

10. Mites (Poultry). Under grant support research has been undertaken on the biology of the mite, Neoschongastia americana, which is a pest of turkeys. Techniques have been developed to culture this mite and study its biology under field conditions. Prelimary research has demonstrated that most infestations occur between the legs and the body. Feeding injury caused by one mite will attract many mites. Only the larval stage is parasitic.

B. Insecticidal and Sanitation Control

1. Mosquitoes (All livestock). At Gainesville, Florida, the search for new and safer insecticides for mosquito control was continued. The most effective material screened as a larvicide was Dursban. Two other materials were slightly less effective. Against adult mosquitoes in laboratory wind-tunnel tests, 7 compounds were more effective than and another 10 equal to the standard, malathion.

In field tests against Aedes, Psorophora, and Culex larvae in small pot-holes, Abate and Dursban gave excellent control at dosages of 0.005 to 0.1 lb/acre. In field tests low-volume aerial applications of naled, naled-malathion mixtures, fenthion, and fenthion-Bay 39007 mixtures were evaulated for the control of adult salt-marsh mosquitoes. All treatments were highly effective in reducing mosquito populations. Some mixtures gave control over longer periods and studies are being undertaken on residual effectiveness of different formulations. A new spray system for applying low-volume applications was developed and evaluated and proved to be as effective as the minispin system used previously. Furthermore, the new system has no bearings to wear out.

In tests with aerosol generators used to disperse insecticides for adult mosquito control from the ground, thermal and non-thermal aerosol generators used to apply malathion, fenthion, and naled were equally effective in killing or controlling caged or natural populations of salt-marsh mosquitoes. Furthermore, water was as good as fuel oil as a diluent for these insecticides when they were applied from non-thermal aerosol generators. With thermal aerosol generators there was no differences in the degree of adult mosquito control when different oils were used as diluents.

Studies were conducted in cooperation with the Department of Defense in Thailand to evaluate the effectiveness of insecticides for the control of Aedes aegypti and Culex quinquefasciatus when applied as adulticides or larvicides. Fogging tests with six insecticides dispersed in fuel oil from a Swingfog pulse jet fogger showed fenthion, Bay 41831 and malathion to give highly effective control. Naled, Bay 39007, and Shering 34615 were also highly effective but required higher concentrations for effective control. As larvicides, Abate and Dursban applied at 0.005 lb/acre and fenthion at 0.05 lb/acre produced almost complete control of Culex quinquefasciatus larvae breeding in ditches and canals under houses. Abate and Dursban gave good residual control as larvicides for Aedes aegypti breeding in concrete water storage jugs.

In Florida resistance to malathion in salt-marsh mosquitoes has been found and confirmed in several counties.

Studies are still in progress to develop new insecticides that can be used as residual sprays where Anopheles mosquitoes have become resistant to currently used materials. In laboratory tests, ten chemicals were highly effective and will be evaluated further. Field tests were conducted in buildings in rice-growing areas in Louisiana naturally infested with Anopheles quadrimaculatus to evaluate a group of organophosphorus and carbamate chemicals as residual insecticides. Two materials - RE-5353 and Schering 34613 - were highly effective, causing 99-100% reduction of adults throughout a 15-18 week test period. Wettable powder formulations of two and emulsion concentrates of three other materials caused reductions of 98-100% on most occasions throughout the same period.

The studies on the use of flame-proofed cheesecloth impregnated with Bay 39007 continued. Treated cloth placed along the edge of the ceiling and in each corner from the ceiling to the floor of buildings containing natural infestations of Anopheles quadrimaculatus produced 98 to 100% control through at least 21 to 22 weeks of aging. On the basis of these successful tests, preliminary evaluations against Anopheles species in Nigeria were conducted in cooperation with the World Health Organization in 1965. Promising results were obtained and further tests are currently under way.

Research was continued at Corvallis, Oregon on the development of insecticides and the evaluation of insecticide resistance. Several new analogs of DDT were tested against Culex tarsalis, but none were highly toxic to resistant strains. Six identified chemicals from western red cedar were not highly toxic to Culex tarsalis, indicating that other compounds in western red cedar must be responsible for the toxicity. In prehatch treatments against snow-water mosquitoes, fenthion and Abate granules and benzene hexachloride wettable powder at dosages of 0.05 to 0.10 lb/acre gave 85% to 90% control. In posthatch treatments granules of Dursban and Abate gave good control of 0.03 lb/acre. Dursban, as an emulsion in fuel oil, at 0.05 lb/acre gave good initial and 18 days' residual control of mosquito larvae in log ponds.

Under contract with the University of California research is underway to evaluate promising insecticides as larvicides and adulticides for irrigation water mosquitoes when applied as low-volume aerial sprays. The distribution and deposit of applications of low volume sprays have been compared to those obtained with conventional spray systems. Preliminary tests against mosquitoes indicate effective control of mosquito larvae with low volumes of organophosphorus insecticides.

2. House Flies (All livestock). At Corvallis, Oreg., basic research was continued on the mode of action of insecticides, mechanisms of insecticide resistance, and means of overcoming the problem of insecticide resistance. To study the genetics of insect resistance to insecticides, several mutants of the house fly were isolated from normal and gamma-irradiated strains reared in the laboratory. The genetic basis of the mutants was analyzed. Most mutants isolated involved wing form, wing positioning, wing venation, eye color or coloration. Four well established, genetically characterized mutant strains, classic wing, stubby wing, dot vein, and white have been extremely useful in studying the genetic basis of insecticide resistance. The resistance spectra of 8 insecticide resistant house fly strains were measured in tests with 15 insecticides. The insecticides included organophosphates, carbamates, chlorinated hydrocarbons, cyclodienes, and a botanical. Resistance to organophosphates and carbamates was associated primarily with fifth chromosomal altered aliesterase genes. Chlorinated hydrocarbon resistance was associated with fifth chromosomal semidominant genes for dehydrochlorination and second chromosomal recessive genes of unknown mechanism. Resistance to cyclodiene insecticides was associated with genes on chromosomes other than the second or fifth.

Research has been continued to develop synergists for organophosphorus and chlorinated hydrocarbon insecticides against insecticide resistant strains. In cooperation with the Pesticide Chemicals Research Branch a large number of candidate synergists are being synthesized or procured and tested.

A factor kdr (for knockdown resistance) conferring resistance to DDT and all other chlorinated hydrocarbons has been verified in 2 house fly strains. The fact that pyrethrins resistance may be due to the same factor suggested that pyrethrins synergists might block resistance associated with this factor. Studies to date have not born out this assumption. Experimental work has failed to demonstrate a metabolic basis for resistance associated with kdr.

Considerable basic research has been developed on esterases in insecticide susceptible and resistant strains of house flies. Disc electrophoresis has been used attempting to separate and concentrate insect esterases. Considerable progress has been made in developing useful techniques and concentrating enzymes. Preliminary research indicates some differences between susceptible and resistant forms. However, considerable research is needed to isolate and study these enzyme systems.

An effort was made to control large populations of flies through the use of dichlorvos resin strips in the septic system and manure disposal wagon of a local slaughter house. A high degree of larval control was obtained. High larval infestations did not develop even though many egg masses were deposited. Little effect was noted on the adult populations since many flies were coming from other areas in the vicinity of the slaughter house.

A laboratory strain of the little house fly was shown to possess a dehydrochlorinative-type factor for resistance to DDT.

At Gainesville, Florida, the search for new insecticides effective in controlling house flies was continued. In laboratory tests, eight experimental insecticides were more effective than the ronnel standard against insecticide susceptible and resistant house flies. Residual tests against house flies in Florida dairy barns were conducted with eight emulsions and wettable powder formulations. Dimethoate residues were the most effective giving satisfactory control for 3 to 6 weeks. Dursban and Mobil MC-A-600 were effective for 1 to 3 weeks and Bay 41831 up to 2 weeks. The remaining compounds produced satisfactory control for less than 1 week and in most instances were ineffective at the 24-hour posttreatment evaluation. Tests were conducted with 19 selected insecticides against natural infestations of house fly larvae in manure under caged poultry to determine their effectiveness as larvicides. All chemicals were applied as emulsions or wettable powder suspensions at 100 mg/ft^2. Shell SD-8448 and SD-8802 gave slightly better control than the dimethoate standard. Other materials were inferior to the standard. None of the compounds tested gave effective control for more than 1 week.

Investigations of physical methods for controlling flies in dairy

operations were continued at Beltsville, Maryland as a cooperative effort of the Entomology, Animal Husbandry, and Agricultural Engineering Research Divisions.

A study was initiated to evaluate the effectiveness of farmstead sanitation practices in reducing fly populations and to determine whether efforts by individual farmers are beneficial, or whether concerted community action is essential. Monitoring surveys were made of native fly populations on neighboring farmstead areas and of the dispersal of marked flies released at various points within the area.

Removal of fly-attractive materials from a dairy complex appeared to reduce the house fly populations by about 1/3. Stable flies were not much affected by barn and corral cleanup. House flies dispersed more rapidly from areas with few breeding sites than from areas with many breeding sites and were more attracted to farms with uncleaned corrals and barns than to farms having daily manure cleanup. Most house fly dispersal occurred within 4 days after adult emergence and was quite rapid to farms within a 1 mile radius of the emergence site.

3. Screw-worm (All livestock except poultry). Of 30 new compounds screened for larvicidal effectiveness at 10, 1.0, and 0.1 ppm in screw-worm larval medium, 7 were highly effective killing all the larvae at 1.0 ppm. None of the compounds screened were effective at 0.1 ppm.

4. Stable Fly (Cattle and horses). Sixty-six compounds were evaluated as stable fly larvicides at Gainesville, Florida. Bayer 24498 and Stauffer N-3794 were better than the standard, Bay 39007. The candidate compounds had LC-50's of 0.62 ppm and 0.72 ppm respectively as compared to 0.94 ppm for Bay 39007. Other highly effective compounds were Bayer 29492, Bayer 48772, and Bayer 48792 with LC-50's ranging from 1.2 to 1.55 ppm.

Sixty-four chemicals were evaluated as stable fly adulticides in contact spray tests in a wind tunnel apparatus using Bay 39007 as a standard. Shell SD-8436 was about equal to the standard in effectiveness. Other promising chemicals were Niagara NIA-10242, Monsanto CP-7768, Niagara NIA-9227, and Shell SD-8448.

Field tests were conducted to compare the effectiveness of fenthion, naled, and malathion in controlling stable flies when these materials were applied by thermal and non-thermal aerosols. Tests were run against caged females with both aerosol generators calibrated to deliver 40 gallons of liquid/hr. Fenthion was slightly more effective than naled and both compounds were about 10 times more effective than malathion. There was no substantial difference in the kill of stable flies treated with thermal or non-thermal aerosols or between fuel oil and water-based formulations in the non-thermal aerosol generator.

A contract was negotiated with the Florida State Board of Health to conduct research on insecticidal methods of controlling stable flies (also called dog flies) under field conditions at their Panama City Research Laboratory. Initial field tests indicated that application rates of naled used as aerosol treatments for mosquito control did not give satisfactory kill of dog flies. Doubling the concentration of naled increased kill to 96%. Preliminary tests applying naled by aircraft at 0.14 and 0.07 pounds per acre gave good to excellent control and further tests are planned.

In Texas, 94 new compounds were screened on cattle in spot tests for repellency and toxicity against the stable fly. Nine of these compounds were class IV toxicants - namely, ENT 25655, 27109, 27300 a, 27324, 27341, 27350, 27382 and 27389. None of the materials tested were effective repellents.

In Texas, large cage tests were conducted to evaluate the effectiveness of various insecticides in backrubbers and as low volume sprays. Backrubbers treated with 1 and 2% ronnel gave only about 50% control of stable flies in 18 hour exposures. Of 12 materials applied at 0.5 - 1.0% at rates of 23 to 46 ml per animal, coumaphos was the most effective, giving complete kills for 3 days. Other materials such as Ciodrin, carbaryl and naled were not completely effective one day after application.

In Texas, seven new materials were evaluated as topical treatments and all gave 100% kill at 0.1 ug/fly, but only one (ENT 20738) was effective at 0.01 ug/fly.

In Texas, extensive tests were conducted to determine the effectiveness of Bakthane (Bacillus thuringiensis) as a feed additive for controlling stable fly breeding in cattle feces. The effectiveness of the soluble portion of Bakthane was greatly reduced in the feces after passage through the ruminant digestive system. The insoluble portion and the whole product were equally effective in preventing stable fly development in the feces.

In Texas, a number of materials were evaluated for toxicity to stable fly larvae. The materials were mixed with manure at different concentrations and larvae added periodically. The most toxic material was dimethoate, a concentration of only 0.5 ppm killing all introduced larvae for a period of 8 to 10 days. Malathion and Abate at 10 ppm were effective for only several days. In similar tests in Nebraska, Bioferm S5-440 was 100% effective at 1.0% and 99% effective at 0.1% but relatively ineffective at concentrations of 0.01% and lower.

5. Cattle Grubs (Cattle). Research was continued in Texas and Oregon, to develop more effective insecticides for the control of cattle grubs and other bots affecting livestock. In Texas, 79 new compounds were screened

for systemic action by giving them orally and subcutaneously at several dosages to guinea pigs infested with larvae of Cochliomyia macellaria and Phormia regina. Twenty-three materials showed systemic activity in one or both types of administration.

In Texas, over 40 systemics were tested for effectiveness against cattle grubs (H. lineatum) on small numbers (3-4) of government cattle. Four were effective in sprays, one in spray and in feed and one in pour on and in feed materials. In large scale tests on cooperator cattle 95 to 100% control of grubs in 4 herds was obtained with 1% sprays of trichlorfon and 8 and 16% pour ons applied at 1 and 0.5 ounces per hundred weight, respectively. Additional tests were conducted in Texas with 10 materials on cattle from Wyoming, which were infested with both species of cattle grubs (H. lineatum and H. bovis). Over 90% control was obtained with 3 materials as sprays, one in feed and one in spray, in feed and by injection.

In Oregon, field tests were conducted on cooperator's cattle to evaluate the effectiveness of Ruelene and Imidan sprays and pour ons of Imidan, Shell SD 8447 and SD 8436. Sprays of 0.375% Ruelene and Imidan gave 99 and 97% grub control respectively. In two tests Imidan pour ons (4 ounces of 1 lb/gal concentrate) gave 90 and 98% control. Other materials were ineffective at even the highest of 3 pour on dosages.

6. Horn Fly. In Texas, tests were conducted to evaluate 9 materials as feed additives for the control of horn flies. Dosages of 10 mg of Shell SD 8447 and Bromophos prevented horn fly larval development in feces. A comparison of the soluble and insoluble fractions of commercially produced Bacillus thuringiensis showed that feeding of the insoluble portion to cattle caused greater mortalities of dipterous larvae in the manure. The endospores and crystalline inclusion were then separated from the insoluble portion and given orally to steers. In tests against horn fly larvae, it was found that spores and inclusion bodies are responsible for a portion of the larvicidal effect. When manure was autoclaved after having been incubated 72 hours the larvicide was destroyed but this was not true for spore-containing manure.

In Texas, 12 materials were applied by sprayer at volumes of 23 to 46 ml/animal. A single application of coumaphos controlled horn flies for 7 days, whereas Ciodrin, carbaryl and naled were effective for only 1 day.

In Texas, pour on applications of 2% coumaphos, 2% fenthion, and 8% trichlorfon (and an 0.75% trichlorfon spray) provided effective control of horn flies for 7 to 13 days in humid coastal areas. Similar treatments were effective for about 21 days in semi-arid areas of Texas. In other field tests a 1% pour on and 0.5% spray of Shell SD 8447 was effective for only 1 to 2 weeks. Special studies were conducted to

determine the number of cattle in herds that should be treated to produce effective control of horn flies. It was found that treatment of one-half of herds gave practically 100% control in 24 hours and good control continued for a week despite heavy rains. Sprays of 0.25% Dursban gave effective horn fly control for about 10 days.

In Mississippi, extensive tests were conducted with several insecticides used in different ways for the control of horn flies. Light spraying in pens with 0.03 to 0.06% coumaphos, 0.25% trichlorfon and 0.025 - 0.1% fenthion gave effective control for about 5 days. Low volumes applied by automatic sprayer of 1.0% coumaphos controlled flies for a week but similar applications of 1.0% Hooker HRS 1422, 0.1% Dursban and 0.1% fenthion were effective for only about 5 days. Backrubbers treated with 0.25, 0.5 and 1.0% fenthion were equally effective for 15 days. Bags containing several insecticidal dusts were tested by placing them in shelters where the cattle would use them. Dusts containing 5% of fenthion, coumaphos or Bayer 9010 provided practically complete control of horn flies for over 4 weeks at which time the tests were terminated. A 0.5% dimetilan dust was effective only 3 weeks.

At Corvallis, Oregon, a study was conducted to evaluate the effectiveness of dust bags treatments of 1.0% dimetilan available to cattle on a free-choice basis for the control of horn flies. Dust bags were installed on a crossbar between two wooden uprights or trees in 5 different pastures located in Corvallis or Summer Lake, Oregon. Results showed successful horn fly control can be attained under certain conditions by the use of free-choice dust bags. Control was excellent in 4 out of 5 herds. In two of these herds dusting on some animals and loss of dust from the bags was considered excessive.

7. Face Fly (Cattle and horses). In Nebraska, extensive bioassy tests were conducted to compare the effectiveness of two Bacillus thuringiensis products against face fly larvae. The products were essentially equal in effectiveness, with both causing complete larval mortality at a concentration of 1.0%. A concentration of 0.1% allowed 25 to 40% of larvae to survive and pupate but no adults emerged from the pupae. Lower concentrations were ineffective.

8. Ticks (Cattle and horses). At Kerrville, Texas, ears of cattle were treated with various formulations and concentrations of 23 insecticides, some of which had been tested the previous year for the control of spinose ear ticks. The effectiveness of the treatments was determined by comparing the numbers of ticks scraped from ears of treated and untreated cattle at 1 week and 1 month posttreatment. Practically all of the insecticides except Dri-die provided a high degree of control at 1 week posttreatment, but only 5% coumaphos dust and 0.1% emulsion of Compound 4072 afforded 100% control of nymphs at 1 month posttreatment.

During the summer of 1965, 3 tests were conducted at Camp Stanely with 2-year-old Hereford heifers naturally infested with lone star ticks to evaluate the efficacy of insecticidal sprays in controlling these ticks. Dursban, bromophos, Shell SD-8447 and 8448, Bay 37341, carbaryl, trichlorfon, Bay 39007, Dowco-175, Banol, and Imidan were applied to cattle at various concentrations in different formulations (1 gal/head) and the effectiveness in controlling lone star ticks was compared to a standard 0.5% toxaphene spray. All insecticides except bromophos (0.375%) and Bayer 37341 (0.1%) were as effective as toxaphene (0.5%) at 1 day posttreatment. A 1 week posttreatment, those treatments affording control equal to or greater than 0.5% toxaphene were: 0.25% Imidan, 0.1% Shell SD-8448, 0.1% Dowco-175, 0.25% Bay 39007, 0.5% Shell SD-8447, 0.1% Dursban, 1% trichlorfon, and 0.5% carbaryl. At 2 weeks posttreatment none of the insecticides gave more than 20% control.

Personnel of the Kerrville, Texas, laboratory continued research on the evaluation of insecticides for the control of Boophilus ticks on cattle. Several hundred candidate toxicants were screened for effectiveness against Boophilus annulatus and/or Boophilus microplus. About 50 compounds proved highly effective when these ticks were dipped in solutions of emulsions of these toxicants. Materials considered highly effective where those that reduced egg laying or egg hatch by at least 90%.

Extensive field tests were conducted in Mexico, to evaluate the effectiveness of promising insecticides as sprays and dips for the control of Boophilus ticks on cattle. Sprays of 0.1% Banol; 0.5% carbaryl; 0.05% carbophenothion; 0.3% Ciodrin; 0.25% coumaphos; 0.15% dioxathion; 0.01% to 0.1%. Dursban; 0.03% to 0.25% Imidan; and 0.1% - 0.45% Shell SD-8447 gave 94% to 100% control of reproduction. Imidan, Dursban, coumaphos, and carbaryl gave complete control of reproduction of one or both species of Boophilus ticks tested. Dipping trials with Dursban and Imidan showed high or complete control of reproduction.

At Corvallis, Oregon field tests were conducted on the effectiveness of three insecticides in controlling ticks, Dermacentor andersoni, on cattle. Newer insecticides were applied to animals as whole body sprays. Toxaphene was applied as a whole body spray and also in a more concentrated form to the tick infested portion of the neck and brisket only. The new materials - Compound 4072 and Ciodrin - gave 82% to 84% control after one week. Whole body sprays of toxaphene were less effective in controlling ticks (89% reductions after 1 week and 0% reduction after 2 weeks) than the more concentrated application applied to the neck and brisket only (97% to 100% reduction after one week, 74% to 97% reduction after 2 weeks, and 29% to 55% reduction after 3 weeks).

9. Ticks (Poultry). In tests at Kerrville, Texas, dust boxes treated with Zytron or Dowco-175 granules had no effect on the numbers of fowl ticks present on infested white leghorn hens.

10. Mites (Poultry). At Corvallis, Oregon several insecticides were bioassayed with the Hanson strain of the northern fowl mite using a topical application method. The Ld-50's were determined as 0.0005% concentration for Shell SD-8447, 0.0033% for Abate, and 0.0072% for malathion.

11. Lice (Poultry). Research on the development of insecticides for the control of poultry lice was continued at Kerrville, Texas. Eighteen selected toxicants were evaluated for poultry lice control by treating birds with acetone solutions of the technical grade chemical at the rate of 40 ml/bird. Seventeen of the candidate toxicants eliminated lice from the birds within 3 to 7 days when applied at spray concentrations of 0.05%, 0.1%, or 0.25% and birds remained free of lice for at least 14 to 28 days. In another test, control of poultry lice by the use of dry insecticides mixed with sand in "dust boxes" was evaluated. One-half pound of granules containing 4.4% Zytron per box reduced lice populations but did not completely eliminate them. In a similar treatment using one pound of granules containing 1% Dowco-175 per box little reduction in numbers of lice occurred.

12. Lice (Sheep and goats). At Kerrville, Texas, Angora goats heavily infested with biting lice were sprayed with Imidan at concentrations of 0.05%, 0.1%, and 0.25%. At 1 day posttreatment some goats in all groups had live lice. At 1 week posttreatment no live lice were found. Four months later at the next shearing all animals were moderately, to heavily infested with lice. Three weeks after shearing, the same goats were sprayed with 0.05%, 0.10%, and 0.25% Shell SD-8447. Live lice were found on goats sprayed with the two lower concentrations at1 day posttreatment and by 1 month infestations were again moderate. With the 0.25% spray, no live lice were seen 1 day posttreatment and very few lice were found 1 month after treatment

On three ranches in Kerrville, Gillespie, and Kimble counties sheep moderately to heavily infested with biting lice were treated with insecticides for louse control. At 4 to 6 weeks after treatment, 0.5% DDT and 0.125% coumaphos afforded complete control; 0.05% Dursban afforded 95% control, and 0.25% Shell SD-8447 and 0.5% carbaryl afforded 70-75% control.

C. Biological Control

1. Mosquitoes (All livestock). At Lake Charles, Louisiana, research on mosquito pathogens as potential biological control agents has been increased. Microsporidian, bacterial, fungal, viral and/or nematode parasites have been found in 35 species of mosquitoes in Louisiana. Microsporidian parasites have been found in about 17 species of mosquitoes including infections of Thelohania, Stempellia, Plistophora and Nosema. Microsporidian parasites have been grouped according to host parasite relationships described by Kellen and co-workers. Transovarial transmission has been demonstrated. Most infections found in field populations of mosquito larvae tend to be of a low order (around 1%). Means and methods of increasing infectivity need to be developed. Corothrella appendiculata,

a very small chaoborid which inhabits tree holes and artificial containers, was observed to be infected with an unknown species of microsporidia. A colony of Culex salinarius infected with Thelohania has been established in the laboratory for experimental purposes. Since only adult females survive from infected egg rafts it is necessary to add males from a non-infected colony to keep the infected colony going.

A species of Spirillum (a bacteria) which invades the hemocoel has been isolated from 12 species of mosquitoes. Infection rates are generally low in field populations. For example, approximately one percent of Culex p. quinquefasciatus collected from a roadside ditch polluted by septic tank effluent were infected.

Coelomomyces infections have been identified in eight species of mosquitoes. An infected colony of Culiseta inornata has been established for laboratory studies and a naturally infested pothole in the field is being studied.

A virus has been identified in Aedes sollicitans, A. taeniorhynchus and A. vexens. A colony of A. taeniorhynchus infected with this virus is being maintained for basic studies of infectivity within and between different species of mosquitoes. Nematodes and internal ciliates have been identified in several species of mosquitoes.

Colonies of Culex p. quinquefasciatus, C. salinarius, Aedes triseriatus, and Culiseta inornata are well established in the laboratory for basic research on biology and pathogens.

At Corvallis, Oregon, research was initiated on microsporidian infections of mosquitoes found in log ponds. Infections were found in Culex pipiens, Culex peus and Culiseta incidens. A technique for determining per os transmission has been developed; however, methods of transmission other than transovarian have not been demonstrated.

At Gainesville, Florida, research on pathogens of mosquitoes has been increased. Microsporidial parasites have been found in Culex salinarius, C. restuans, Anopheles quadrimaculatus, and A. crucians. It has been impossible to infect healthy larvae with these parasites so research has been initiated to elucidate life cycle and biology of the parasites. Fungi of the genus Coelomomyces and Achlya have been isolated from several species of mosquitoes as well as several species of bacteria. A microsporidian has been found infecting a species of chironomid.

Under contract with the Fresno Laboratory of the California State Public Health Department through the University of California at Berkeley is researching pathogens as biological control agents. Various species of bacteria -- Bacillus cereus, B. thuringiensis, B. sphaericus, Aeromonas hydrophila, Pseudomonas sp., Flavobacterium sp., and Achromobacter sp.; fungi -- Spicaria farinosa and Coelomomyces psorophorae; microsporidia:

and viruses have been identified as pathogens in western species of mosquitoes. Host-parasites relationships and methods of infectivity are now under study.

Under contract funds with the McNeese State College at Lake Charles, Louisiana research is being conducted on predators of mosquito larvae. Studies are in progress on insects and fish as predators of mosquito larvae and have included Mollienesia latipinna and Cyprinodon variegatus and species of the family Corixidae. Some observations indicate corixids may lay their eggs on crawfish. This is of special interest in relation to Psorophora confinnis in Louisiana rice fields where many owners are alternating between rice and crawfish crops.

2. Houseflies (All livestock). A grant with the University of California has been negotiated to study the effect of predacious mites on populations of flies breeding in manure. Progress to date has been limited to identifying mite species and determining density of mites in various fly breeding sites.

A PL-480 project in Korea is making excellent progress in identifying species of beetles present in dung and defining their role in the dispersion of dung with related effects on fly breeding. Progress to date is mainly in determining species, their distribution, biology, and life cycles.

3. Imported Fire Ant (All livestock). Under PL-480 support, research was continued on parasites of the imported fire ant in Uruguay. The distribution of imported fire ant colonies has been studied and recorded. Data has been collected on various forms of arthropods found in ant mounds. Of particular interest have been studies on an ant, Labauchena daguerri, which parasitises the imported fire ant. Laboratory and field studies have shown that L. daguerri is apparently dependent upon the imported fire ant for survival and weakens imported fire colonies in the field.

4. Face Fly (Cattle and horses). At Lincoln, Nebraska, adults of Aleochara tristis, a European parasite of the face fly, were received early in 1965 and successfully colonized in the laboratory. Between May 24 and August 19 over 40,000 adults were released in 40-acre pasture occupied by a small herd of cattle. Parasitized face fly pupae were found in 15 days after the first adults were released. Peak parasitism of about 12% obtained during late July and early August declined rapidly thereafter to zero by late August. Parasitized pupae were recovered up to 1 1/4 miles from the release pasture within 2 months after adult releases started.

Extensive tests were conducted to study the behavior of A. tristis under laboratory and field conditions. It was found that the newly

hatched tristis larvae preferred 3- and 4-day-old face fly pupae to those 1-and 2-day-old, but parasitism was relatively low even with favorable ratios of larvae to pupae. In host preference studies with other species of dipterous pupae, face fly were preferred over other species. However, in the absence of the face fly, pupae of Orthellia caesaviane were more acceptable than other dipterous pupae, with house flies being next in order. Very little parasitism of the stable fly (Stomoxys calcitrans) and the horn fly (Haematobia irritans) occurred.

Laboratory tests in Nebraska showed that larvae and pupae of A. tristis cannot survive 1-week exposure to temperatures of 10° to 20° F. Survival was good for 1 to 2 weeks at 45° to 50° F but poor after 4 to 5 weeks, and good for 3 weeks at 55° F but only fair after 5 weeks. Old pupae survived 45° to 55° F storage much better than maturing larvae and young pupae. However, under field conditions adult tristis survived in good numbers over winter despite the lack of snow and minimum temperatures of 18° F.

In course of field studies on A. tristis another similar appearing species, A. bimaculata, was discovered and found to be parasitizing several species of Sarcophaga and Orthellia but only rarely the face fly. The habits of bimaculata are similar to those of tristis.

In Nebraska adult face flies were observed to hibernate in the same barns for the fourth consecutive year. First hibernation was noticed on Sept. 25 and calculations suggest hibernation may be related to average daily temperatures below 65° F. No adult flies or larvae were noted in the field after mid-October.

D. Insect Sterility and Other New Approaches to Control

1. Mosquitoes (All livestock). At Gainesville, Florida, research was continued on the evaluation of the sterility principle for control of mosquitoes. The effect of varying dosages of gamma radiation on fecundity and fertility of Aedes aegypti treated in the pupal stage was determined. Females were completely sterilized at 3,000r, while males were completely sterilized at 10,000 r. Male Culex p. quinquefasciatus were almost completely sterilized at doses of 7,000 to 12,000. Males irradiated at 8,500 and 10,000r were less competitive than untreated males in mating. Males of C. p. quinquefasciatus were effectively sterilized by dusting with apholate powder. The sterility induced was permanent and the sterilized males were competetive with untreated males in mating untreated females.

Two sterile male release studies were made with a semi-isolated population of Anopheles quadrimaculatus. Males were sterilized by exposure to

residual deposit of tepa. Although some sterility was induced in the natural population (up to 42%), no reduction in the numbers in the population occurred. A bait of apholate was placed in natural resting stations of A. quadrimaculatus in this same area in another experiment. Although a high degree of sterility occurred in mosquitoes feeding on the bait, there was little sterility in the natural population and no reduction in its size.

The colony of Aedes aegypti which developed resistance to apholate through laboratory selection is being maintained. Previously reported resistance was confirmed and the level has continued to increase. Tests indicated that this apholate-resistant colony was not resistant to tepa. Studies have been initiated to determine the mechanism involved in resistance to apholate.

2. House Flies (All livestock). Research was continued at Gainesville, Florida, on the development of sterilization techniques for house fly control. Six hundred and thirteen chemicals were screened for chemosterilant activity and 67 of these caused complete sterility in adults. Twenty-five of the chemicals also caused complete sterility in males. Tests were conducted to determine whether chemicals causing sterility in house flies affected sperm motility. All of the chemosterilants found in screening tests to date were evaluated and none had any effect on sperm motility. One hundred and seventy two chemosterilants were evaluated for sterilizing effects when they were applied to house fly rearing medium containing third instar larvae. Eleven of these chemicals caused complete sterility by preventing oviposition or egg hatch. House fly pupae immersed in solution of hempa or 2,4-diamino-6-(2-furyl)-s-triazine for periods of 5 to 30 minutes were sterilized. However, the sterilizing dose was probably acquired mostly through adult contact with treated pupal cases during emergence since washing pupae after treatment reduced sterility to low levels (35% to 46%). Hempa caused a reduction in cholinesterase activity in in vivo and in vitro tests with house flies.

Tests were conducted on Grand Turk Island in the Bahamas to evaluate the effectiveness of an integrated program for the control of house flies. The approach consisted of making insecticide and chemosterilant applications to larval breeding sites as well as releasing adult flies that had been sterilized by gamma irradiation. High population reductions were achieved through the application of larvicides and chemosterilant baits and there was a high degree of sterility in the population of house flies remaining on the island. The release of sterile insects appeared to increase the level of sterility in the natural population but did cause sufficiently to eradicate the population. Either the number of sterile insects released was too low or their behavior and dispersion was abnormal. Research has been undertaken to study dispersion of released house flies on the

island and the density of the natural population existing on the island. House fly populations were reduced in numbers by the integrated control scheme to approximately 1,000,000.

At Corvallis, Oregon, research was undertaken on methods of sterilizing the little house fly. Tepa, metepa, apholate, hempa, and hemel were evaluated in the laboratory as chemosterilants. Residual treatments of tepa 0.1-1.0 mg/ft^2 sterilized these flies. Metepa and hempa were somewhat less effective, and apholate failed to sterilize at 50 mg/ft.^{2} When fed to adult flies, tepa was again the most effective, producing a high degree of sterility at concentrations of 0.01 to 0.1% in adult food. The other materials were about equally effective. In experiments on mating competitiveness, males treated with hempa and metepa competed well with untreated males. There was no great margin between dosages causing complete sterility, especially of females, and those causing some mortality. Pupae were unaffected when dipped in 0.05 to 5% ethanol solutions of metepa.

3. Screw-worm (all livestock except poultry). Of 646 compounds screened as chemosterilants, 29 caused sterility in 1 or both sexes of screw-worm flies when fed to adults. Evaluation of chemosterilants passing screening tests showed that some compounds sterilized also when administered as topical treatments, some sterilized only 1 sex, and some sterilized both male and female flies.

Additional tests were performed with 3 chemosterilants (ENT-50838, 50716, 50781) previously reported to equal or surpass radiation in their effectiveness in achieving sterility of screw-worm flies. In test of sexual aggressiveness of males, harassment of females by chemosterilized males was very nearly equal to that by untreated males. In other tests, chemosterilized males survived at least 90% as well as fertile controls.

ENT-50781 effectively sterilized males 0-1, 3-4, or 6-7 days old. Sterility of females 0-1 day old was high, but that of females 3-4 and 6-7 days old was complete, or nearly complete. Males offered ENT-50781-treated food for 16 hours were nearly sterile. Sterility after 20- and 24-hour intervals was equal and essentially complete. Males treated orally with ENT-50781 were ground, extracted with chloroform, and fed to new males. Enough active chemosterilant was recovered to give rise to moderate and slight sterility 24 and 72 hours, respectively, after treatment.

Tests were conducted to find methods of administration of ENT-50838 other than oral or topical treatments of adults that were effective. ENT-50838 sterilized when pupae were immersed in solutions and the emerging flies were in contact with the residual chemosterilant on the shed puparia for more than 48 hours. Complete sterility was induced in males by tarsal contact for 4 hours with a residual deposit of 20 mg/ft^2 of ENT-50838 on glass. The deposit was moderately effective after 2 weeks and ineffective after 5 weeks.

In tests of mating competition in a laboratory room, males sterilized topically by ENT-50838 were at least as competitive as untreated males. However, males sterilized by tarsal contact competed for females about half as well as untreated males.

Untreated males or females acquired the chemosterilant and a consequent high degree of sterility by bodily contact with flies of the opposite sex treated topically 5 days earlier with ENT-50838. However, males treated by injection did not transfer the chemosterilant by contact to previously mated females 5 days later.

ENT-50838 topically sterilized both sexes 0-7 days old essentially with equal facility. Males treated topically with ENT-50838 showed no loss of sterility 3 weeks later. There was no recovery from sperm damage induced 48 hours earlier in 5-day-old males injected intrathoracically with ENT-50838. It was found that incompletely sterile populations of males were composed of some males with 100% dominant lethals in the sperm and others with fewer than 100% dominant lethals. ENT-50838 was equally effective in topically sterilizing virgin and mated females.

Other chemosterilants were also tested. ENT-51086 did not greatly affect male sexual vigor adversely in tests of sexual aggressiveness of males. However, it adversely affected the mating vigor of males by a factor of about 1.5-2.0 in tests of mating competition. ENT-50451 applied topically induced dominant lethals equally well in sperm prior to ejaculation and sperm stored in spermathecae.

A possible basis for the preferential action of some chemosterilants according to mode of administration was sought. A blood pH of 6.0-6.8 and a mid- and hindgut pH of >4.7->7.0 might explain the greater vulnerability to degradation of some aziridinyl compounds, which are acid-sensitive, by the oral than the topical route.

4. Stable Fly (Cattle and Horses). In Texas, exposure of stable flies on deposits of 10 to 375 mg/ft^2 failed to induce complete sterility. In additional tests, topical application 0.125-30 ug/fly of tepa gave almost complete sterility of males but they were slightly less competitive in mating than normal flies. The treatments did not appear to reduce fecundity in crosses with untreated females.

In Texas, studies were conducted on the mode of action of the chemosterilant, apholate, on stable flies. Preliminary studies using radioactive Thymidine indicated an interference in young stable flies of the synthesis of DNA by apholate. The interference appeared to be in the incorporation of Thymidine into the primordial egg cells.

5. Horn Fly (Cattle). At Kerrville, Texas, research was continued on the development of new methods for the control of horn flies on cattle. An electro-chemical device was developed which provided effective control of

horn flies and completely avoided the risk of contamination of the cattle, milk or milking equipment. The device uses BLB ultraviolet fluorescent lamps installed behind insecticide impregnated gauze. Horn flies are attracted to the light, contact the treated gauze and die in a few minutes. When operated during total or semi-darkness these devices reduced larvae horn fly populations by 90 to 95% within 7 to 10 days and maintained effective control thereafter. Additional studies are planned to determine the efficacy of covering entrances to barns to create a semi-darkness environment in which the units would be effective at all hours of the day.

6. Tsetse Flies. Under a PASA agreement with AID research was continued in Salisbury, Rhodesia in cooperation with the Agricultural Research Council of Central Africa on the feasibility of the sterile male technique for control of tsetse flies. Following successful research developing effective methods of chemosterilizing 2 species of tsetse, Glossina morsitans and G. pallidipes, field tests have shown that sterile males are competitive with normal males in small cage tests and that sterile males disperse and survive as well as normal males under natural field conditions. An island has been selected for a small field experiment on the release of sterile males. Approximately 9-months data has been accumulated on population levels, dynamics, survival and birth rates to serve as a basis for designing a release experiment. Progress had been made in attempts to develop techniques for establishing self-sustaining colonies of tsetse in the laboratory or in cages in field environments; however, a productive self-sustaining colony for use in a release experiment or program has not been developed to date.

E. Insecticide Residue Determinations

1. Residue studies. In Texas further studies were conducted to determine the residues in animal tissues produced from contact with back rubbers treated with ronnel. Analyses have been made previously of ronnel residues in body tissues of cattle following use of a home-made back rubber constructed by covering a chain with several piles of burlap. A new type of back rubber is now on the market consisting of a stainless steel cylinder about 4 inches in diameter and 10 feet long, suspended in a horizontal position about 3 1/2 feet from the ground, with a strip of heavy cotton cloth attached to the bottom of the cylinder. The cylinder is filled with an oil solution of the insecticide, which escapes through holes in the underside to keep the suspended cloth saturated. Since this appeared to disperse more insecticide than the back rubbers used in earlier tests, determinations were made of ronnel residues in body tissues following the use of the new device. One group of eight cattle were allowed to use a back rubber charged with a 1% solution of ronnel, and another similar group with 2% solution. One control animal used a back rubber charged with the oil solvent only. The animals were forced to walk under the rubbers four times daily for 28 days. Since the earlier tests had shown that residues of ronnel were preponderantly in the fat,

the only tissues analyzed in this test were the renal and omental fat. The amount of ronnel found in the fat after 2 weeks use of the back rubber ranged from 0.006 to 0.031 ppm and after 4 weeks use the range was 0.008 to 0.097 ppm. Two and three weeks after the back rubbers were removed there was no ronnel detectable in the fat except for one animal in which 0.001 ppm was found two weeks after removal.

In Texas a study was made to determine whether small amounts of nonachlor in the feed of dairy cows would cause contamination of the milk. A Holstein cow was fed 2 ppm of nonachlor in its feed for 14 days. Milk samples were collected at intervals during the feeding period and after feeding had ceased. During the feeding of nonachlor the amount of residue found in the milk rose to a maximum of 0.255 ppm (milk adjusted to 4% butterfat). Ten days after feeding had ceased the residue had fallen to 0.022 ppm of nonachlor in the milk. Since residues had not yet been completely eliminated, additional samples were taken for analysis to determine the time required for complete elimination of the nonachlor.

In Texas analyses were made of the omental, renal, and subcutaneous fat of cattle following a single spray or dip treatment with 0.05% Dursban. Five weeks after treatment, the fat of the cattle that had been dipped contained from about 0.001 to 0.007 ppm of Dursban, while the fat of cattle that had been sprayed contained 0.001 to 0.003 ppm. Analyses also were made of fat from cattle that had received three dippings at 2-week intervals in 0.05% Dursban. Ten weeks after the last dip the Dursban residues in the fat ranged from 0 to 0.002 ppm. Another study was made to determine the residues in various body tissues one week after a calf that had been sprayed with 0.25% Dursban. The amounts found in the various tissues were muscle 0.007, liver 0, heart 0, kidney 0.002, brain 0.002, omental fat 0.60, renal fat 0.67, and subcutaneous fat 0.71 ppm.

In Washington extensive residue studies were conducted of plants in the Burns Tussock Moth Project area where DDT had been applied by helicopter at the rate of 0.75 lb/ acre. Residues calculated on a dry-weight basis were as follows: Sedge 9.3 to 261 ppm DDT (average 69), lupine 44 to 220 ppm (average 88), sagebrush 25 to 143 ppm (average 56). Four months after the application of DDT had been made vegetation samples were again taken and analyzed with the following results (dry-weight basis); sedge 5.2 to 22 ppm (average 13), lupine 3.2 to 17 ppm (average 13), sagebrush 3.2 to 12 ppm(average 5.9). Samples of fish and aquatic invertebrate organisms also were obtained from within the treated area and analyzed for DDT and its metabolites. The invertebrate pretreatment sample (from a creek bottom) had a residue of 0.9 ppm and another (a drift sample at the mouth of a creek)contained 101.0 ppm of DDT. Fish samples collected in June and July from the area sprayed in May and June contained residues ranging from 0.14 to 2.1 ppm. Adipose tissue of cattle from the Tussock Moth Control area also was analyzed for DDT

and its metabolites. The cattle were sampled in the fall following the spring treatment. Pretreatment samples of the adipose tissue showed from 0.02 to 4.4 ppm of combined DDT and its metabolites. The range and average of combined DDT and its metabolites found in the samples taken in the fall from cattle in several states in the area were as follows: California 6.3 to 16 ppm (average 12), Idaho 12 to 86 ppm (average 44), Oregon 1.7 to 7.7 ppm (average 5.2). Forty water samples were also collected and analyzed for DDT and its metabolites. Pretreatment samples showed no indication of DDT or metabolites. Twenty-eight samples of water from the treated area contained residues of < 0.1 to 0.2 ppb of o,p'-DDT and <0.2 to 0.7 ppb of p,p'-DDT. Three samples from the treated area contained 0.2 to 0.7 ppb of o,p'- and 0.7 to 3.6 ppb of p,p'-DDT. Neither DDE nor TDE was detected in any of the samples. One sample from an area where insecticides had been spilled contained a residue of 3.5 ppb o,p'- and 19.0 ppb p,p'-DDT. This work was in cooperation with the Forest Service.

In Texas a dairy cow was dipped twice with a one-week interval, in a 0.22% solution of As_2O_3. Milk samples were taken for analysis from 1 to 14 days after the last dipping. The arsenic residues found in the samples one day after dipping amounted to 0.02 ppm; by the 10th day after the last dipping the residues were no longer detectable. Beef cattle were similarily treated and animals were slaughtered and tissue samples taken for analysis 2, 3, 4, and 7 days after the last treatment. Analyses are being made of various tissues and organs from these animals.

In Texas, analyses were made of omental fat samples from beef cattle at weekly intervals after they had been dipped in 0.22% ronnel emulsion. Fat samples taken after 1 week showed about 0.1 ppm. Residues declined to 0.002 ppm after 3 weeks and none were detectible after 4 weeks. Further studies were made to determine the residues produced by feeding ronnel to cattle at the rate of 8.76 mg/k per day for 14 days. The average residue at the end of the feeding period was 6.6 ppm. Residues decreased to only 0.011 ppm in 21 days and had completely dissipated in 35 days. In another test, residues were determined in cattle that had access (free choice) for 11 weeks to a mineral supplement containing 6% ronnel, 75% salt, and 19% other materials. The average intake of ronnel over the 11-week period was 4.1 mg/kg/day. Analyses showed an average residue of 0.89 ppm at the end of the feeding period. Low but detectible residues were still present in some but not all samples after 35 days.

In Texas, studies were conducted by veterinarians of the Animal Disease and Parasite Research Division to determine residues in the omental fat of sheep that had been dipped weekly for 9 weeks in 0.5% bromophos emulsion. Fat samples taken 1 day after the last dipping contained from 5.0 to 14.0 ppm. Residues of 0.07 to 0.43 ppm were still present 22 days after the final treatment.

2. Toxicity studies. Research was continued in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals to livestock.

Tests were conducted to determine the effects of single treatments of 15 insecticides on the plasma tocopherol levels in calves. No positive trends were noted other than a general depletion with passage of time. Fourteen of the insecticides caused no outward sysmptoms of poisoning at the initial concentration testsed. However, four proved highly toxic at the highest of two test concentrations.

Studies were conducted to compare the effects of four chemosterilants on poultry. Chickens given 10.0 mg/kg orally daily of tepa or metapa became moribund and were destroyed after 24-32 doses (cumulative totals of 126-190 mg/kg/bird). All birds showed a marked loss in weight, ataxia and a characteristic squatting position when at rest. At necropsy the livers were friable and fatty degeneration was evident. The spleens were small and pale and the lungs were congested.

Chickens given daily doses of 5, 10, or 20 mg/kg of apholate or 50 mg/kg of hempa showed no signs of poisoning after 41 doses. At necropsy the livers, spleens and testes of the apholate-treated birds were smaller than normal but no gross lesions were noted.

Extensive studies were conducted to determine the toxicity of Abate to sheep and cattle. Sheep consuming from 1.9 to 2.9 mg/kg daily in water (20 times normal expected consumption)showed no ill effects and only slight ChE depression after 109 days. Cattle consuming 1.1 to 1.4 mg/kg showed no clinical evidence of damage after 4 months but ChE was reduced to 67-79% of normal.

Several series of tests were conducted to determine the toxicity of parathion to sheep. Dipping in 0.005% parathion caused no toxic symptoms but some of the sheep sprayed with 0.01 and 0.025% exhibited moderate to severe effects and a few animals died. Sheep tolerated and consumed feed containing 50 ppm of parathion without ill effects for 21 days and ChE was not greatly depressed. Sheep on feed containing 100 ppm of parathion were not visibly poisoned but their ChE was lowered to 22-47 percent of normal.

In tests with phosdrin, dermal applications of 0.05% caused no toxic symptoms to sheep and a concentration of 0.025% had no ill effects on cattle, but ChE was depressed in both kinds of animals. Oral doses of 5 mg/kg to sheep and 0.5 mg/kg to cattle caused no toxic symptoms but reduced ChE moderately. A few of many sheep sprayed with 0.25 or 0.5% carbophenothion were visibly poisoned but the majority exhibited no ill effects despite marked reduction of ChE. None were affected by 0.1% dips.

In Texas as in previous years a large number (over 60) new candidate insecticides were tested for toxicity to cattle, calves, and sheep when administered orally or dermally as sprays or dips. Toxicities ranged from impossibly dangerous to reasonably safe. Several of the safer materials posses excellent insecticidal activity and further studies will be undertaken to relate this to a safer dosage for possible practical control use in the event of registration for use on livestock.

F. Attractants.

1. Screw-worm (All livestock except poultry). In Texas 211 chemicals were screened as screw-worm attractants. Of these, 8 were found to compete with the standard liver bait. However, further evaluation eliminated all but ENT-5963 and ENT-28236. These compounds have received limited field tests but further testing is required as the data acquired from these tests are inconclusive.

The male screw-worm pheromone extract has been fractionated by Pesticide Chemicals Research Branch and the active material has been concentrated. Virgin female flies responded to this material even when it was diluted 200 times. In order to facilitate the identification of this material, a change in collecting technique was initiated in the hope that the number of extraneous materials in the extract could be reduced. The male pheromone was collected by passing the air from the cage containing males over activated charcoal. Hexane extracts of the charcoal have produced the same type of response by unmated female flies as that observed when they were exposed to the male pheromone extracts collected by the cold trap method. Both "searching motions" and aggressive male-type mating "strikes" were discerned.

Studies on the response of female screw-worm flies to selected odors revealed that habituation or a permanent waning of a response without reinforcement occurred in this insect. The most vigorous response occurred during the initial exposure period of 15 minutes. The response declined thereafter with little or no response being observed after test periods of 45 minutes. Tests made after periods of recovery where the odor was removed and then re-exposed to the flies determined that at least 2 hr was needed between experiments. After this period of time had elapsed, the flies responded almost as vigorously as they did in the first test period. Studies on the effect of temperature on the response of screw-worm flies to odors established that 64° F was the temperature threshold of response. No fly activity was observed at lower temperatures.

2. House Flies. At Gainesville, Florida, a method was developed to screen various chemicals as attractants (olfactory stimulation) for house flies. Various tests showed that edamin could be used as a standard, although its ability to attract flies is minimal. Of 113 chemicals that have been screened none have shown a high degree of attractancy.

Certain chemicals produced an anomaleus avoidance or repellency response.

Tests were run on certain chemosterilants to determine if they were repellent to house flies. Apholate was somewhat repellent; metapa and hempa may be considered highly repellent. Hempa did not attract or repel house flies by olfaction so that repellency may be tactile or gustatory.

Other tests showed that the age or condition of ovaries influenced the responsiveness of female house flies to ammonium hydroxide. Response differed with differing concentrations and a concentration of 1.0 N ammonium hydroxide was preferred over all other.

Research was continued on the sex pheromone reported earlier in the house fly. Considerable emphasis has been given to cooperative work with chemists of the Pesticide Chemicals Research Branch attempting to isolate active components or fractions of the pheromone. Isolation has not been accomplished to date. It was confirmed that the presence of the pheromone in female flies was age related. No house fly sex pheromone-like activity was present in benzene extracts of female or male black blow flies, female false stable flies, female or male stable flies, or female or male little house flies. To date clear demonstrations of activity have been observed only with extracts of female and male horn flies, and female house flies.

Approximately 90 miscellaneous compounds were screened as attractants for the little house fly in field tests near natural populations. None of the compounds showed an attraction of these flies or other muscid, calliphorid, and sarcophagid flies in the area.

G. Insect Vectors of Animal Diseases

1. Anaplasmosis (Cattle). In 1965, tests in Mississippi were designed to reflect the importance of horse flies and mosquitoes as vectors of bovine anaplasmosis. A group of 3 susceptible and 1 carrier animals were exposed continuously outdoors to attack by horse flies and mosquitoes. A similar group was exposed in a screened cage which excluded horse flies. Two of the 3 susceptible animals in the continous exposure test developed anaplasmosis and the other was a suspect, whereas none of the caged animals developed the disease. Similar additional groups of animals were used.

As a part of the anaplasmosis studies, the seasonal incidence of horse flies and mosquitoes was studied by means of animal baited traps, collection from bait animals, and direct counts on test animals. Animal baited traps operated from 5:00 PM to 7:00 AM (May 21-July 27) yielded over 17,000 mosquitoes of 14 species, with Psorophora confinnis composing 60% and Anopheles quadrimaculatus 25%. Only 300 horse flies were

captured with T. vittiger schwardti, T. fuscicostatus, and T. lineloa, composing 66%, 14%, and 14% respectively. Peak populations occurred in late May and early June. Similar animal traps operated from 7:00 AM to 5:00 PM caught very few (199) mosquitoes, 70% of which were A. quadrimaculatus. Only 400 horse flies were captured, 80% being vittiger schwardti and 13% lineola.

Collections from a tethered bait horse and steer totaled about 5,000 on each animal, with peak abundance occurring late in May. Both collections were composed of about 75% vittiger schwardti, 8% lineola, 4.5-7.0% fuscicostatus, and small numbers of 9 other species. Counts of house flies on the anaplasmosis test animals varied from 3 to 13.6 at various observations and the peak occurred from June 14 to 21. Stable fly populations varied from 12 to 14, with the peak occurring from June 14 to 28. Horn fly population was never numerous, ranging from 42 to 94 with the peak occurring from June 1 to 10. As in 1964, all evidence suggests the horse flies are the primary vector of anaplasmosis and that mosquitoes apparently are not involved.

At Beltsville, Maryland, studies on the transmission of bovine anaplasmosis were continued, but at a much reduced level because of other research on disease transmission. Transmission trials and attempts to develop "infected colonies" of ticks have been unsuccessful. Colonies of ticks are being established from infected areas.

2. Equine Piroplasmosis. At Beltsville, Maryland efforts were continued during the last year to develop an "infected colony" of Dermacentor nitens. Two attempts to infect normal colony D. nitens with Babesia caballi by allowing the ticks to feed on horses infected by blood passage were apparent failures as the resulting progeny did not transmit the disease to susceptible horses.

Two positive transmissions of Babesia caballi were obtained with the progeny of field collected ticks from two different premises in Dade County, Florida. In the first transmission trial, a splectomized horse developed acute EP 15 days after tick exposure and died 9 days later. At the time of death none of the ticks were sufficiently engorged to produce eggs so these ticks, 79 females and 87 males, were transferred to the ears of another B. caballi infected horse. Only 4 engorged females were recovered, and the resulting progeny were lost due to incubator failure. In the second transmission trial, another horse developed acute EP 18 days after tick exposure. This horse survuved the acute course of the disease. Thirty-nine engorged females were recovered, 10 of which were utilized for cytological study and the remainder were held for colonization.

A third positive transmission was obtained by exposure of an uninfected horse to approximately 1,000 progeny of ticks fed on an EP infected horse. The horse developed acute EP 18 days after tick exposure and survived the acute course of the disease. Ten engorged females were recovered. Three were used for cytological study and the remaining ticks were held for

colonization. Providing this colony can be maintained and strengthened, a Babesia caballi infected colony of D. nitens will be established. Two positive serial transmissions indicate that the parasite and this particular strain of D. nitens are well adapted to each other.

In one attempt to infect Dermacentor nitens with Babesia equi, a splenectomized horse was infested with approximately 20,000 larvae from the normal colony. On the 6th day after infestation the horse was inoculated with B. equi infected blood from a carrier animal. Acute B. equi infection developed 5 days later and death occurred on the 9th day after inoculation. None of the ticks had fed sufficiently for oviposition. Molting nymphs and flat adults were transferred to another horse infected with B. equi, however, none of the ticks attached.

Engorged females from test horses infected with B. equi were examined systematically to determine the development of B. caballi during the preoviposition and oviposition periods. Gut smears were made daily as ticks dropped from the infected horses and were continued at daily intervals until the tick died or until oviposition was completed. Immediately upon death, each specimen was dissected and gut, malpighian tubule, ovary, and salivary gland tissues were subjected to routine cytological investigation. Giemsa's stain was used for all observations. Developing stages of B. caballi were first found 24 hours after the ticks dropped from the infected host. Development appears to continue for 5 to 7 days when the parasites become evident in the ovaries and in ova which are apparently mature. These preliminary studies have not completely elucidated the life cycle of B. caballi in D. nitens. However it appears that at least two stages of multiple division occur within the tick; the first occurring in the epithelial cells of the gut wall, and the second occurring in the cells of the malpighian tubules and/or in ovarian cells.

3. Bluetongue (Sheep and Cattle). Studies have been continued on the biology of insect vectors of bluetongue disease of sheep and the role of insects in the transmission of the disease. This research is conducted in cooperation with the Animal Disease and Parasite Research Division at Denver, Colorado. Research has shown that bluetongue virus multiplies in Culicoides variipennis, a proven vector of the disease. Research has also demonstrated that cattle can be involved in the transmission cycle since C. variipennis that have fed on infected cattle (infection caused artificially be innoculation or naturally by insect bite) transmitted the disease to sheep. Using an electron-microscope, studies have been undertaken to study the biology of bluetongue disease in the salivary glands of C. variipennis. When fed on Blucine (egg-attenuated), vaccinated sheep picked up enough virus to allow multiplication of this virus in the insect host so that the insects transmitted a more virulent form of the disease than was evident in the vaccinated animal.

Preliminary studies have developed a technique for feeding Culicoides variipennis through a membrane on fluids containing a known amount of

virus and shown that flies can become infected with this technique.

Biological studies of field and laboratory populations of Culicoides have been continued. An overwintering larval population at Hudson, Colorado, was studied. It consisted of Culicoides variipennis, C. crepuscularis and a new species. This larval population completed its emergence by June and by this time a continuous summer population had begun.

PUBLICATIONS--USDA AND COOPERATIVE PROGRAMS

Basic Biology, Physiology and Nutrition

Baumhover, A. H., and G. E. Spates, Jr. 1965. Artificial selection of adult screw-worms for extended survival without food and water. J. Econ. Entomol. 58: 645-49.

Chapman, H. C., and D. B. Woodard. 1965. Blood feeding and oviposition of some floodwater mosquitoes in Louisiana: laboratory studies. Mosq. News 25: 259-62.

Crystal, M. M, and H. H. Meyners. 1965. Influence of mating on oviposition by screw-worm flies (Diptera: Calliphoridae). J. Med. Entomol. 2: 214-16.

Eye, R. L., and G. C. LaBrecque. 1966. Sexual acceptability of laboratory strains of male house flies in competition with wild strains. J. Econ. Entomol. 59: 538-40.

Graham, O. H., and M. A. Price. 1966. Some morphological variations in Boophilus annulatus microplus (Canestrini) (Acarina: Ixodidae) from Northern Mexico. Ann. Entomol. Soc. of Amer. 59: 450-52.

Harris, R. L., E. D. Frazar, P. D. Grossman, and O. H. Graham. 1966. The mating habits of the stable fly. J. Econ. Entomol. 59: 634-36.

Hightower, B. G., P. B. Davis, A. H. Baumhover, and O. H. Graham. 1966. Seasonal abundance of screw-worms in Northern Mexico. J. Econ. Entomol. 59: 416-20.

Hoyer, R. F. 1966. Some new mutants of the house fly (Musca domestica L.) with notations of related phenomena. J. Econ. Entomol. 59: 133-37.

Hoyer, R. F., F. W. Plapp, Jr., and R. D. Orchard. 1965. Linkage relationships of several insecticide resistance factors in the house fly. Entomol. Exp. Appl. 8: 65-73.

Killough, R. A., J. G. Hartsock, W. W. Wolf, and J. W. Smith. 1965. Face fly dispersal, nocturnal resting places, and activity during sunset as observed in 1963. J. Econ. Entomol. 58: 711-15.

Killough, R. A., and E. S. McClellan. 1965. Face fly oviposition studies. J. Econ. Entomol. 58: 716-19.

Morgan, N. O., and C. D. Schmidt. 1966. The pH tolerance of horn fly larvae. J. Econ. Entomol. 59: 222-23.

Murvosh, C. M.,and C. W. Thaggard. 1966. Ecological studies on the house fly. Ann. Entomol. Soc. Amer. 59: 533-47.

Roberts, R. H. 1966. A technique for rearing the immature stages of Tabanidae (Diptera). Entomol. News, Vol. LXXVII, No. 3.

Roberts, R. H. 1965. A stable trap for sampling insects affecting cattle. Mosq. News 25: 281-85.
Smith, C. N. 1965. Need for standard and other strains of Aedes aegypti for research. World Health Organ./Vector Control/190.65. Seminar on the Ecology, Biology, Control and Eradication of Aedes aegypti 117-20.
Woodard, D. B., and H. C. Chapman. 1965. Blood volumes ingested by various pest mosquitoes. Mosq. News 25: 490-91.

Conventional Insecticide Control Methods

Bigley, W. S. 1966. The inhibition of cholinesterase and aliesterase in parathion and paraoxon poisoning in the house fly. J. Econ. Entomol. 59: 60-65.
Brady, U. E. 1966. A continuous exposure technique for determination of insecticide resistance in house flies. J. Econ. Entomol. 59: 764-65.
Claborn, H. V., H. D. Mann, I. L. Berry, and R. A. Hoffman. 1965. Comparisons of residues in milk resulting from two types of spray applications of DDT, Shell Compound 4072 and ronnel. J. Econ. Entomol. 58: 922-23.
Drummond, R. O. 1965. Further evaluation of animal systemic insecticides, 1964. J. Econ. Entomol. 58: 773-76.
Drummond, R. O., S. E. Ernst, C. C. Barrett, and O. H. Graham. 1966. Sprays and dips of Shell Compound 4072 to control Boophilus ticks on cattle and larve of the screw-worm. J. Econ. Entomol. 59: 395-400.
Drummond, R. O., and O. H. Graham. 1965. Systemic insecticides in livestock insect control. Vet. Rec. 77: 1418-20.
Drummond, R. O., and O. H. Graham. 1966. Dermal application of ten systemic insecticides for the control of common cattle grubs. J. Econ. Entomol. 59: 723-26.
Drummond, R. O., and J. G. Medley. 1965. Field tests with insecticides for the control of ticks on livestock. J. Econ. Entomol. 58: 1131-36.
Drummond, R. O.,and J. M. Ossorio. 1966. Additional tests with insecticides for the control of the tropical horse tick on horses in Florida. J. Econ. Entomol. 59: 107-10.
Drummond, R. O., T. M. Whetstone, and S. E. Ernst. 1965. Control of cattle grubs with coumaphos applied by sprayer and spray-dip machine. J. Econ. Entomol. 58: 1017-18.
Drummond, R. O., T. M. Whetstone, and S. E. Ernst. 1966. Control of ticks on cattle with toxaphene applied by power sprayer and spray-dip machine. J. Econ. Entomol. 59: 471-72.
Gahan, J. B., B. M. Glancey, and C. S. Lofgren. 1965. New larvicides that show promise against mosquitoes. Proc. New Jersey Mosq. Exterm. Assoc. 156-59.
Gjullin, C. M., and G. W. Eddy. 1965. Low volume airplane sprays for the control of mosquitoe larvae. Mosq. News 25: 442-47.
Glancey, B. M., C. S. Lofgren, J. Salmela, and A. N. Davis. 1965. Low volume aerial spraying of malathion for control of adult salt-marsh mosquitoes. Mosq. News 25: 135-37.

Harris, R. L., E. D. Frazar, and O. H. Graham. 1966. Resistance to ronnel in a strain of horn flies. J. Econ. Entomol. 59: 387-90.
Harris, R. L., O. H. Graham, and W. C. McDuffie. 1965. Resistance of livestock insects to insecticides in the United States. Agr. and Vet. Chem. 6: 78-81.
Hoffman, R. A., I. L. Berry, and O. H. Graham. 1965. Control of flies on cattle by frequent, low-volume mist spray applications of Ciodrin R. J. Econ. Entomol. 58: 815-17.
Hoyer, R. F.,and F. W. Plapp, Jr. 1966. A gross genetic analysis of two DDT-resistant house fly strains. J. Econ. Entomol. 59: 495-501.
Ivey, M. C., H. V. Claborn, R. A. Hoffman, O. H. Graham, J. S. Palmer, and R. D. Radeleff. 1966. Residues of Shell Compound 4072 in the body tissues of sprayed cattle. J. Econ. Entomol. 59: 379-82.
Lewis, L. F., D. M. Christenson, and G. W. Eddy. 1965. Control of mosquito larvae in Willamette Valley, Oregon log ponds. Mosq. News. 25: 400-02.
Lofgren, C. S., N. Pennington, and W. Young. 1966. Evaluation of insecticides against two species of Culex mosquitoes on Okinawa. Mosq. News 26: 52-59.
McDuffie, W. C.,and D. E. Weidhaas. 1965. The future outlook for new and improved materials and methods for mosquito control. Mosq. News 25: 85-91.
Morgan, N. O.,and R. R. Blume. 1966. Laboratory technique of detecting tolerance or susceptibility of horn flies to insecticides with modified WHO test kit. J. Econ. Entomol. 59: 749-50.
Morgan, P. B., G. C. LaBrecque, and H. G. Wilson. 1966. Tests with larvicides for the control of house flies (Musca domestica L.) in poultry houses. Florida Entomol. 49: 91-93.
Mount, G. A. 1965. Use of the WHO tsetse fly kit for determining resistance in the stable fly. J. Econ. Entomol. 58: 794-96.
Mount, G. A., J. B. Gahan, and C. S. Lofgren. 1965. Evaluation of insecticides in the laboratory against adult and larval stable flies. J. Econ. Entomol. 58: 685-87.
Nelson, T. E., and Knut M. R. Bertun. 1965. Synergism of malathion against the northern fowl mite. J. Econ. Entomol. 58: 1117-18.
Plapp, F. W., Jr., and Homer H. C. Tong. 1966. Synergism of malathion and parathion against resistant insects: phosphorus esters with synergistic properties. J. Econ. Entomol. 59: 11-15.
Plapp, F. W., Jr., R. D. Orchard, and J. W. Morgan. 1965. Analogs of parathion and malathion as substitute insecticides for the control of resistant house flies and the mosquito, Culex tarsalis. J. Econ. Entomol. 58: 953-56.

Insect Parasites, Predators, and Pathogens

Cole, M. M. 1965. Biological control of ticks by use of Hymenopterous parasites. World Health Organ./EBL/ 43.65.
Gingrich, R. E. 1965. Thelophania tabani n. sp., a microsporidian from larvae of the black horse fly, Tabanus atratus Fabricius. J. Invert. Pathol. 7: 236-40.

Insect Sterility and other New Approaches to Control

Crystal, M. M. 1966. Sexual sterilization of screw-worm flies by a peroral chemosterilant: quantitative aspects and relation to pre-treatment starvation. J. Econ. Entomol. 59: 580-85.

Crystal, M. M. 1966. Some structure-activity relationships among aziridinyl antifertility agents in screw-worm flies. J. Econ. Entomol. 59: 577-80.

Crystal, M. M. 1965. Sexual sterilization of insects by aerosol administration of alkylating agents. J. Econ. Entomol. 58: 678-80.

Drummond, R. O., J. G. Medley, and O. H. Graham. 1966. Engorgement and reproduction of lone star ticks, Amblyomma americanum (L.) treated with gamma radiation. Intern. J. Radi. Biol. 10: 183-88.

Eddy, G. W., A. R. Roth, and L. R. Abrahamsen. 1965. Sterilant effect of some materials on Aedes aegypti (L.) feeding on treated mice. Mosq. News 25: 169-71.

Fye, R. L., G. C. LaBrecque, and H. K. Gouck. 1966. Screening tests of chemicals for sterilization of adult house flies. J. Econ. Entomol. 59: 485-87.

Glancey, B. M. 1965. Hempa as a chemosterilant for the yellow-fever mosquito (Aedes aegypti (L.)) (Diptera: Culicidae). Mosq. News 25: 392-96.

LaBrecque, G. C., P. B. Morgan, D. W. Meifert, and R. L. Fye. 1966. Effectiveness of hempa as a house fly chemosterilant. J. Med. Entomol. 3: 40-43.

LaChance, L. E., and J. G. Riemann. 1964. Cytogenetic investigation on radiation and chemically induced dominant lethal mutations in oocytes and sperm of the screw-worm fly. Mutation Res. 1: 318-33.

McDuffie, W. C. and D. E. Weidhaas. 1965. The future outlook for new and improved materials and methods for mosquito control. Mosq. News 25: 85-91.

Smith, C. N. 1965. Research needs and priorities: genetic control, including sterile male technique. World Health Organ/Vector Control/ 190.65 pp. 255-58.

Weidhaas, D. E. 1965. Research on radioisotopes and radiation in insect control related to the development of the sterile-male technique. 5th Inter-Am. Symp. Peaceful Appl. Nucl. Energy: 189-95.

Attractants and Repellents

Fletcher, L. W., J. J. O'Grady, Jr., H. V. Claborn, and O. H. Graham. 1966. A pheromone from male screw-worm flies. J. Econ. Entomol. 59: 142-43.

Gjullin, C. M., J. O. Johnsen, and F. W. Plapp, Jr. 1965. The effect of odors released by various waters on the oviposition sites selected by two species of Culex. Mosq. News 25: 268-71.

Insect Vectors of Livestock Diseases

Anthony, D. W. and T. O. Roby. 1966. The experimental transmission of bovine anaplasmosis by three species of North American ticks. Amer. J. Vet. Res. 27: 191-98.

Jones, R. H. 1965. Epidemiological notes: incidence of Culicoides variipennis in an outbreak of bluetongue diesease. Mosq. News 25: 217-18.

PUBLICATIONS - STATE EXPERIMENT STATIONS and COOPERATIVE PROGRAMS

Basic Biology, Physiology, and Nutrition

Messersmith, D. H. 1965. Culicoides (Diptera: Ceratopogonidae) associated with poultry in Virginia. Mosq. News 25: 321-4. (Va.)

Pitts, C. W., and T. L. Hopkins. 1965. Lipid composition and hibernation of face flies, Musca autumnalis. Bull. Entomol. Soc. Amer. 11: 159. (Kan.)

Conventional Insecticide Control Methods

Adkins, T. R. Jr., and J. A. Hair. 1965. Absence of residues in milk after dimethoate and malathion were applied with backrubbers to dairy cattle. J. Econ. Entomol. 58: 155. (S.C.)

Bruce, W. N., R. P. Link, and G. C. Decker. 1965. Storage of heptachlor epoxide in the body fat and its excretion in milk of dairy cows fed heptachlor in their diets. J. Agr. and Food Chem. 13(1): 63-67. (Ill.)

Cheng, T. H., A. A. Hower, and R. K. Sprenkel. 1965. Oil-based and water based Ciodrin sprays for fly control on dairy cattle. J. Econ. Entomol. 58: 910-13. (Pa.)

Dodson, R. C., and D. P. Sanders. 1965. Low-volume, high-concentrate spraying for horn fly and face fly control of beef cattle. J. Econ. Entomol. 58: 379. (Ind.)

Hair, Jakie A., and T. R. Adkins, Jr. 1965. Dusting stations and cable backrubbers as self-applicatory devices for control of the face fly. J. Econ. Entomol. 58: 39-41. (S.C.)

Knapp, F. W. 1965. Free-choice feeding of ronnel mineral block and granules for face fly, horn fly and cattle grub control. J. Econ. Entomol. 58: 836-38. (Ky.)

Riehl, L. A., H. W. Lambright, and P. D. Ludwig. 1965. Area population control of heel flies by Ruelene pour-on application annually to cattle. J. Econ. Entomol. 58: 1-4. (Calif.)

Deema, P., E. C. Naber, and G. W. Ware. 1965. Residues in eggs from vaporizing insecticide tablets. J. Econ. Entomol. 58: 904-906. (Ohio)

Riehl, L. A., D. C. Addis, J. B. Burgess, and A. S. Deal. 1965. Trials of Ruelene for cattle grub control in southern California. J. Econ. Entomol. 58: 361-2. (Calif.)

Sherman, M. and C. H. Komatsu. 1965. Toxicity to fly larvae of droppings from chicks reared on insecticide-treated feed. J. Econ. Entomol. 58: 203-206. (Hawaii)

Sherman, M. 1965. The effectiveness of insecticides administered orally to the fowl as a deterrent to the breeding of flies in droppings. Proc. Hawaiian Entomol. Soc. XIX (1): 111-117. (Hawaii)

Smith, C. T., F. R. Shaw, D. L. Anderson, R. A. Callahan, and W. H. Ziener. 1965. Ronnel residues in eggs of poultry. J. Econ. Entomol. 58: 1160-1161. (Mass.)

Treece, R. E., and G. W. Ware. 1965. Lindane residues on alfalfa and in milk. J. Econ. Entomol. 58: 218-19. (Ohio)

Turner, Jr., E. C. 1965. Area control of the face fly using self-applicating devices. J. Econ. Entomol. 58: 103-5. (Va.)

Insect Parasites, Predators, and Pathogens

Hair, J. A., and E. C. Turner, Jr. 1965. Attempted propagation of Nasonia vitripennis on the face fly. J. Econ. Entomol. 58: 159-60. (Va.)

Jackson, John O., and B. H. Wilson. 1965. Observations on some predators of horse fly and deer fly (Diptera: Tabanidae) eggs in Louisiana. Ann. Entomol. Soc. of Amer. 58: 934-35. (La.)

O'Donnell, A. E., and R. C. Axtell. 1965. Predation by Fuscuropoda vegetans (Acarina: Uropodidae) on the house fly (Musca domestica). Ann. Entomol. Soc. Amer. 58: 403-4. (N. C.)

AREA NO. 14. INSECTS AFFECTING MAN, HOUSEHOLDS, AND INDUSTRIAL ESTABLISHMENTS

Problem. Insects, ticks, and mites are responsible for widespread human misery and certain insects cause heavy losses of food and materials in households and industrial establishments. Many of the same or closely related insects which affect man are also important pests of livestock, thus research on insects in relation to man and to livestock is mutually advantageous. Certain arthropods are vectors of major diseases which annually cause the deaths of millions of humans. Mosquitoes, for example, transmit malaria, dengue, encephalitis, yellow fever, and filariasis. Some of these diseases, as well as other arthropod-borne diseases, occur and are potentially serious problems in the United States but most of them are of more concern in other parts of the world where troops and civilian personnel of the United States are maintained. The military agencies have for many years depended on the research competence in agriculture for answers to their military insect and insect-borne disease problems. Attacks by insects, ticks, and mites frequently interfere with farm and forest work, reduce or destroy the value of recreation areas, and even make certain areas uninhabitable. Property values are often depressed and development prevented by hordes of annoying pests. Mosquitoes, bed bugs, and fleas are frequently serious annoyances in homes. Other household insects are of economic importance in homes and industrial establishments because they damage foods, fabrics, and other materials, causing losses of millions of dollars annually. There is a great need for safe, economical insecticides and satisfactory methods for their application that could be used quickly and effectively to control local infestations or outbreaks of pests that annoy man in the field or at home, especially where there are threats of disease epidemics. Improved means for controlling mosquitoes, sand flies, gnats, the imported fire ant, and similar pests should receive particular attention. More efficient repellents are needed to protect humans, particularly when other means of control cannot be employed. Special efforts should be made to develop systemic materials which when taken orally would repel or prevent insects from biting. Sanitation, habitat management, and other noninsecticidal methods of control should be reappraised, and biological control, especially with insect pathogens, needs to be fully explored. New approaches to control including chemosterilants, antimetabolites, attractants, and radiation require intensive investigation. Studies should be undertaken on the biology, ecology, physiology, and genetics of many important pests affecting man and the household in order to find weak points in their life cycles which might be utilized to improve control efficiency.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving basic and applied research on the biology and control of insects and related arthropods affecting man,

households, and industrial establishments including studies on mosquitoes, gnats, house flies, human lice, bed bugs, fleas, ticks, mites, cockroaches, ants, and other pests. The Federal scientific effort devoted to research in this area totals 24.2 scientific man-years of which 5.2 is concerned with basic biology, physiology, and nutrition; 8.8 with conventional insecticidal control methods; 1.7 with insect predators, parasites, and pathogens; 3.9 with insect sterility and other new approaches to control; 3.6 with attractants and repellents; and 1.0 with program leadership.

The major portion of the program is conducted at Gainesville, Fla.; the remainder at Corvallis, Oreg.; Fresno, Calif.; Lake Charles, La.; and Beltsville, Md. Close cooperation and evaluation of research needs and data are maintained with the Department of Defense through the Armed Forces Pest Control Board concerning studies on insects important to military personnel. Research funds supporting 3 scientific man-years have been transferred from the Department of Defense to support research in this area. Cooperation is maintained with the World Health Organization on studies for developing new insecticides and other methods of control of insects affecting man. The World Health Organization provides financial support (1/2 scientific man-year) for studies at Gainesville, Fla., on the development of residual insecticides for mosquito control.

Federal support devoted to research grants, contracts, and extended cooperative agreements totals 6.9 scientific man-years per 3 years of which 1.4 is devoted to basic biology, physiology, and nutrition; 3.0 to conventional insecticide control methods; 0.8 to insect parasites, predators, and pathogens; 1.2 to insect sterility and other new approaches to control; and 0.5 to attractants and repellents. Grants, contracts, and cooperative agreements are located at the following institutions: University of California, Davis; University of California, Berkeley; University of California, Riverside; Florida State Board of Health, Panama City; University of Florida, Gainesville; McNeese State College, Lake Charles, La.; University of Southwestern Louisiana, Lafayette; and Virginia Polytechnic Institute, Blacksburg.

Additional research is carried out under grants supported by P.L. 480 funds including S9-ENT-7, "Investigations of natural enemies of ants," Ministeria de Ganaderie y Agricultura, Montevideo, Uruguay; F4-ENT-6, "Studies for the control of house flies and mosquitoes by means of chemosterilants in Egypt," Department of Entomology, University of Cairo, Egypt; and A10-ENT-11, "Action of repellents on mosquitoes, fleas, ticks, and mites," Department of Entomology, Israel Institute for Biological Research, Nes-Ziona, Israel.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 21.2 professional man-years is devoted to this area of research.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Gainesville, Fla., bacterial infusions of hay and fresh grass have been found to stimulate growth in Aedes aegypti, Anopheles quadrimaculatus, Aedes triseriatus, Aedes taeniorhynchus, and Culex quinquefasciatus. In preliminary tests these infusions have also been found to stimulate oviposition and egg hatch and induce autogeny. There is also some indication that they contain substances which attract mosquitoes. Research is now under way to determine whether bacteria or chemical substances are responsible for these stimulations and responses.

Tests conducted to determine whether females of Culex p. quinquefasciatus mated more than once, showed that females did not re-mate and one mating was sufficient to satisfy mating requirements of females.

At Lake Charles, La., studies on the biology of mosquitoes were continued. The blood feeding and oviposition behavior of Aedes canadensis was studied. one blood meal was required to produce one batch of eggs. In the laboratory, females would not take more than 3 blood meals or lay more than 3 egg batches. None of the eggs hatched, showing that this species is univoltine in this area. In an attempt to compare the degree of hatching of eggs of some floodwater species of Aedes with the number of times the eggs were flooded with water, open and screened enclosures were built over breeding sites on marsh areas. In one of four sets of open and screened enclosures, it appeared that all of the hatchable eggs had been depleted after numerous floodings over one year. The remaining screened enclosure continued to produce sizeable but smaller broods of larvae than the open enclosures.

Research has been continued under two contracts at the University of Southwestern Louisiana and McNeese State College. Light trap collections have shown the relative abundance and dispersion of different species of mosquitoes. Correlations of the occurrence of floodings and high tides were made and showed that the numbers of Aedes sollicitans trapped during the second quarter of 1965 were higher than the numbers of Culex salinarius and this trend was reversed during the 3rd and 4th quarters. Data on temperature, rainfall, and the direction and velocity of wind are being gathered to correlate with abundance and dispersal of various species. The seasonal activity of several important species of mosquitoes was determined in two areas in Louisiana. Aedes sollicitans and Culex salinarius were present and active throughout the year except for short periods of no significant rain or high tides. Culiseta inornata was caught only in the cooler months; Mansonia perturbans and Psoropha confinnis were active only in the warm months. Anopheles quadrimaculatus was absent from one area and relatively scarce in the other. Culex quinquefasciatus was found only in one spot near a residential area where a few premises were not well cared for.

At Corvallis, Oreg., studies were continued on the basic biology of mosquitoes. Exposure of Culex quinquefasciatus females to low levels of light during the night influenced their behavior and response to attractants the following day. Surveys on mosquito abundance indicated that Culex tarsalis and Culex peus were less abundant in the Willamette Valley in 1965 than in previous years. During the fall of the past 3 years, larvae of Aedes increpitus have been collected rather widely in the flood plain of the Willamette River indicating that this increpitus strain is well-adapted to the region.

2. House Flies. At Corvallis, Oreg., research was continued on the basic biology of the little house fly. Field collected little house flies were labeled with P^{32} and released for migration studies. Flies dispersed throughout a poultry farm and fur farm within 24 hours. Studies were conducted on their mating behavior. Males were shown to be capable of successfully fertilizing at least 7 females each. Preliminary tests indicate females may mate more than one time. Wild-collected females deposited eggs that ranged from 72 to 82% viable. Excess numbers of males caged with females did not increase female mortality or decrease fecundity or fertility.

3. Eye Gnats. A grant to the University of Florida was negotiated to study the basic biology of eye gnats and to evaluate attractants and sterilization as methods of control. Current research has concentrated on basic biology and initiating research techniques. Colonies of eye gnats have been established and rearing techniques including nutritional requirements evaluated and improved. Facilities have been obtained for studying temperature and light requirements and attractants. Adult surveys have shown population trends in the Gainesville area and suitable field sites for field studies.

B. Insecticidal and Sanitation Control

1. Mosquitoes. At Gainesville, Fla., the search for new and safer insecticides for mosquito control was continued. The most effective material screened as a larvicide was Dursban. Two other materials were slightly less effective. Against adult mosquitoes in laboratory wind-tunnel tests, 7 compounds were more effective than and another 10 equal to the standard, malathion.

In field tests against Aedes, Psorophora, and Culex larvae in small pot-holes, Abate and Dursban gave excellent control at dosages of 0.005 to 0.1 lb/acre. In field tests low-volume aerial applications of naled, naled-malathion mixtures, fenthion, and fenthion-Bay 39007 mixtures were evaluated for the control of adult salt-marsh mosquitoes. All treatments were highly effective in reducing mosquito populations. Some mixtures gave control over longer periods and studies are being undertaken on residual effectiveness of different formulations. A new spray system for applying low-volume applications was developed and evaluated and proved to be as effective as the minispin system used previously. Furthermore, the new system has no bearings to wear out.

In tests with aerosol generators used to disperse insecticides for adult mosquito control from the ground, thermal and non-thermal aerosol generators used to apply malathion, fenthion, and naled were equally effective in killing or controlling caged or natural populations of salt-marsh mosquitoes. Furthermore, water was as good as fuel oil as a diluent for these insecticides when they were applied from non-thermal aerosol generators. With thermal aerosol generators there was no difference in the degree of adult mosquito control when different oils were used as diluents.

Studies were conducted in cooperation with the Department of Defense in Thailand to evaluate the effectiveness of insecticides for the control of Aedes aegypti and Culex quinquefasciatus when applied as adulticides or larvicides. Fogging tests with six insecticides dispersed in fuel oil from a Swingfog pulse jet fogger showed fenthion, Bay 41831, and malathion to give highly effective control. Naled, Bay 39007 and Shering 34615 were also highly effective but required higher concentrations for effective control. As larvicides, Abate and Dursban applied at 0.005 lb/acre and fenthion at 0.05 lb/acre produced almost complete control of Culex quinquefasciatus larvae breeding in ditches and canals under houses. Abate and Dursban gave good residual control as larvicides for Aedes aegypti breeding in concrete water storage jugs.

In Florida resistance to malathion in salt-marsh mosquitoes has been found and confirmed in several counties.

Studies are still in progress to develop new insecticides that can be used as residual sprays where Anopheles mosquitoes have become resistant to currently used materials. In laboratory tests, ten chemicals were highly effective and will be evaluated further. Field tests were conducted in buildings in rice-growing areas in Louisiana naturally infested with Anopheles quadrimaculatus to evaluate a group of organophosphorus and carbamate chemicals as residual insecticides. Two materials were highly effective, causing 99-100% reduction of adults throughout a 15-18 week test period. Wettable powder formulations of two and emulsion concentrates of three other materials caused reductions of 98-100% on most occasions throughout the same period.

Studies on the use of flame-proofed cheesecloth impregnated with Bay 39007 were continued. Treated cloth placed along the edge of the ceiling and in each corner from the ceiling to the floor of buildings containing natural infestations of Anopheles quadrimaculatus produced 98 to 100% control through at least 21 to 22 weeks of aging. On the basis of these successful tests,preliminary evaluations against Anopheles species in Nigeria were conducted in cooperation with the World Health Organization in 1965. Promising results were obtained and further tests are currently underway.

Research was continued at Corvallis, Oregon, on the development of insecticides and the evaluation of insecticide resistance. Several new analogs

of DDT were tested against Culex tarsalis, but none were highly toxic to resistant strains. Six identified chemicals from western red cedar were not highly toxic to Culex tarsalis, indicating that other compounds in western red cedar must be responsible for the toxicity. In prehatch treatments against snow-water mosquitoes, fenthion and Abate granules and benzene hexachloride wettable powder at dosages of 0.05 to 0.10 lb/acre gave 85 to 90% control. In posthatch treatments, granules of Dursban and Abate gave good control at 0.03 lb/acre. Dursban, as an emulsion in fuel oil, at 0.05 lb/acre gave good initial and 18 days' residual control of mosquito larvae in log ponds.

Under a contract with the University of California, research is under way to evaluate promising insecticides as larvicides and adulticides for irrigation water mosquitoes when applied as low-volume aerial sprays. The distribution and deposit of applications of low volume sprays have been compared to those obtained with conventional spray systems. Preliminary tests against mosquitoes indicate effective control of mosquito larvae with low volumes of organophosphorus insecticides.

2. House Flies. At Corvallis, Oreg., basic research was continued on the mode of action of insecticides, mechanisms of insecticide resistance, and means of overcoming the problem of insecticide resistance. To study the genetics of insect resistance to insecticides, several mutants of the house fly were isolated from normal and gamma-irradiated strains reared in the laboratory. The genetic basis of the mutants was analyzed. Most mutants isolated involved wing form, wing positioning, wing venation, eye color, or coloration. Four well established, genetically characterized mutant strains, Classic wing, stubby wing, dot vein, and white, have been extremely useful in studying the genetic basis of insecticide resistance. The resistance spectra of 8 insecticide resistant house fly strains were measured in tests with 15 insecticides. The insecticides included organophosphates, carbamates, chlorinated hydrocarbons, cyclodienes, and a botanical. Resistance to organophosphates and carbamates was associated primarily with fifth chromosomal altered aliesterase genes. Chlorinated hydrocarbon resistance was associated with fifth chromosomal semidominent genes for dehydrochlorination and second chromosomal recessive genes of unknown mechanism. Resistance to cyclodiene insecticides was associated with genes on chromosomes other than the second or fifth.

Research has been continued to develop synergists for organophosphorus and chlorinated hydrocarbon insecticides against insecticide resistant strains. In cooperation with the Pesticide Chemicals Research Branch, a large number of candidate synergists are being synthesized or procured and tested.

A factor kdr (for knockdown resistance) conferring resistance to DDT and all other chlorinated hydrocarbons has been verified in 2 house fly strains. The fact that pyrethrins resistance may be due to the same factor suggested that

pyrethrins synergists might block resistance associated with this factor. Studies to date have not born out this assumption. Experimental work has failed to demonstrate a metabolic basis for resistance associated with kdr.

Considerable basic research has been developed on esterases in insecticide susceptible and resistant strains of house flies. Disc electrophoresis has been used attempting to separate and concentrate insect esterases. Considerable progress has been made in developing useful techniques and concentrating enzymes. Preliminary research indicates some differences between susceptible and resistant forms. However, considerable research is needed to isolate and study these enzyme systems.

An effort was made to control large populations of flies through the use of dichlorvos resin strips in the septic system and manure disposal wagon of a local slaughter house. A high degree of larval control was obtained. High larval infestations did not develop even though many egg masses were deposited. Little effect was noted on the adult populations since many flies were coming from other areas in the vicinity of the slaughter house.

A laboratory strain of the little house fly was shown to possess a dehydrochlorinative-type factor for resistance to DDT.

At Gainesville, Fla., the search for new insecticides effective in controlling house flies was continued. In laboratory tests, 8 experimental insecticides were more effective than the ronnel standard against insecticide susceptible and resistant house flies. Residual tests against house flies in Florida dairy barns were conducted with 8 emulsion and 1 wettable powder formulations. Dimethoate emulsion residues were the most effective, giving satisfactory control for 3 to 6 weeks. Dursban and Mobil MC-A-600 were effective for 1 to 3 weeks and Bay 41831 up to 2 weeks. The remaining compounds produced satisfactory control for less than 1 week and in most instances were ineffective at the 24 hour post-treatment evaluation. Tests were conducted with 19 selected insecticides against natural infestations of house fly larvae in manure under caged poultry to determine their effectiveness as larvicides. All chemicals were applied as emulsions or wettable powder suspensions at 100 mg/ft^2. Shell SD-8448 and SD-8803 gave slightly better control than the dimethoate standard. Other materials were inferior to the standard. None of the compounds tested gave effective control for more than 1 week.

3. Stable Fly. Sixty-six compounds were evaluated as stable fly larvicides at Gainesville, Fla. Bayer 24498 and Stauffer N-3794 were better than the standard, Bay 39007. The candidate compounds had LC-50's of 0.62 ppm and 0.72 ppm respectively as compared to 0.94 ppm for Bay 39007. Other highly effective compounds were Bayer 29492, Bayer 48772, and Bayer 48792 with LC-50's ranging from 1.2 to 1.55 ppm.

Sixty-four chemicals were evaluated as stable fly adulticides in contact spray tests in a wind tunnel apparatus using Bay 39007 as a standard. Shell SD-8436 was about equal to the standard in effectiveness. Other promising chemicals were Niagara NIA-10242, Monstanto CP-7768, Niagara NIA-9227, and Shell SD-8448.

Field tests were conducted to compare the effectiveness of fenthion, naled, and malathion in controlling stable flies when these materials were applied by thermal and non-thermal aerosols. Tests were run against caged females with both aerosol generators calibrated to deliver 40 gallons of liquid/hr. Fenthion was slightly more effective than naled and both compounds were about 10 times more effective than malathion. There was no substantial difference in the kill of stable flies treated with thermal or non-thermal aerosols or between fuel oil and water-based formulations in the non-thermal aerosol generator.

A contract was negotiated with the Florida State Board of Health to conduct research oninsecticidal methods of controlling stable flies (also called dog flies) under field conditions at their Panama City Research Laboratory. Initial field tests indicated that application rates of naled used as aerosol treatments for mosquito control did not give satisfactory kill of dog flies. Doubling the concentration of naled increased kill to 96%. Preliminary tests applying naled by aircraft at 0.14 and 0.07 pounds per acre gave good to excellent control and further tests are planned.

4. Cockroaches. At Gainesville, Fla., research was continued on the development of new, more effective insecticides for cockroach control. Laboratory evaluations included tests with contact sprays, residual deposits, and dust formulations. In contact spray tests 32 compounds produced 75 to 100% mortality within 24 hr at a 2% concentration. Five of these compounds were highly effective at 0.5%. Of materials tested in residual treatments Dursban and Mobil MC-A-600 were highly effective. In the interest of finding better insecticidal dusts for cockroach control, several carbamate insecticides--Mobil MC-A-600, Bay 39007, and carbaryl--were evaluated and their effectiveness compared to malathion and diazinon. Mobil MC-A-600 was the most effective material followed by Bay 39007. Carbaryl was not highly effective.

It has been reported that an anthelmintic known as Parvex is toxic to German cockroaches. In laboratoty tests, residues of this material were not very effective in killing German cockroaches. It produced high knock-down initially, but cockroaches tended to recover.

Comparative evaluations of 5 bait-toxicant formulations and a trichlorfon standard bait were made against German cockroaches in laboratory tests. All of the baits produced less than 100% kill in one day, but 100% mortality occurred in 3 days with the Bomyl bait, 8 and 12 days with a bait containing 1 and 2% General Chemical GC-9160, 12 days with a bait containing 1%

General Chemical GC-987, and 15 days with a 2% mirex bait. The trichlorfon bait produced complete mortality after 9 days.

Studies are being continued on insecticide resistance in cockroaches primarily to attempt to develop resistance to new insecticides in laboratory colonies and to study cross-resistance. To date, resistance has not been found in colonies subjected to selection with residual deposits of Bay 39007, diazinon, or fenthion.

5. Body Lice. Research was continued at Gainesville, Fla., to develop more effective insecticides for the control of human body lice. Eighty new candidate synergists were tested in combination with one or more of 4 insecticides--carbaryl, Bay 39007, Carbanolate, and Zectran--against body lice in laboratory tests. None of the synergists were highly effective, but 19 of them showed some synergism when combined with two or three of the insecticides. Another new synergist, Tropital, was tested in combination with pyrethrins and carbaryl. Tropital was less effective than the standard synergists, sulfoxide or piperonyl butoxide.

Promising insecticides found in primary screening tests were evaluated in secondary tests. Four compounds including ENT-27180, 27124, 27126, and 27192-A were more effective than the standard malathion. When louse powders containing bromophos, carbaryl and malathion were compared, bromophos and carbaryl were almost equal in residual effectiveness, but slightly less effective than malathion. Bromodan, a commercial dust, was not effective against DDT resistant body lice. Furthermore, it stained cloth. Three candidate louse toxicants--Bay 39007, Carbanolate, and Hooker HRS-1422--were compared with standard insecticides--malathion and carbaryl--in tests on research subjects at Camp LeJeune, N. C., in cooperation with personnel of the Navy Medical Field Research Laboratory. Bay 39007 was the most effective of the three candidate materials, but less effective than carbaryl or malathion. Subjects in the tests were under medical supervision and no reactions to the test compounds were noted. Field tests were conducted on inmates of the Republic of Korea, Army Disciplinary Center near Seoul, Korea, with two carbaryl louse powders and malathion. Results were somewhat erratic with less control than expected since some subjects apparently changed clothes. There were no observed toxic or irritating effects from the powders to the test subjects, power-dust operators, or supervisors. Urinalyses for 1-naphthol were negative.

Colonies of resistant body lice are being maintained for research purposes. The search for systemic insecticides and repellents was continued. Of 25 compounds evaluated, only one was effective as a systemic toxicant.

6. Mites, Ticks, and Fleas. Research on insecticidal methods of controlling fleas was continued at Gainesville, Fla. Of 59 candidate chemicals evaluated as systemic toxicants for oriental rat fleas, 4 caused complete mortality to some lots of fleas within 5 hours after treatment without noticeably

affecting the guinea pigs to which the chemicals were administered. The compounds and the lowest effective dosages were: General Chemical GC-8266, 10 mg/kg; Azodrin, 10 mg/kg; 0,0-dimethyl 1-acetoxyethyl-2,2,2-trichlorophosphonate, 100 mg/kg; and Spencer S-6900, 200 mg/kg.

In order to evaluate a practical means by which systemic toxicants might be used to control fleas, several systemically active chemicals were incorporated into the food of rats and offered them either with or without other sources of food. Rats were infested with oriental rat fleas. Mirex, fenthion, diazinon, and trichlorfon in the food were effective in killing fleas infesting the rats.

Research was continued on the evaluation of residual insecticide treatments in controlling fleas. Approximately 25 chemicals passed the criterion for further evaluation. Of these materials, 12 were highly effective in laboratory tests causing 90 to 100% mortality of fleas for a period of at least 24 weeks.

Tests were conducted at Camp LeJeune, N. C., in cooperation with personnel of the Navy Medical Field Research Laboratory to study the effectiveness of 10 organophosphorus and 3 carbamate insecticides against natural infestations of lone star ticks, Amblyomma americanum. Treatments were applied at a dosage of 2 pounds active ingredient in 50 gallons of spray/acre. All the insecticidal sprays caused reductions of adult and nymphal ticks ranging from 86 to 100% initially and a high reduction for 7 to 35 days. Dust formulations of DDT and carbaryl (2 lb/acre) were also evaluated and gave 87 and 56%, respectively, initial reduction. Further tests are planned to determine minimum effective dosages.

7. Bed Bugs. At Gainesville, Fla., research was continued on the development of more effective insecticides for bed bug control. Twenty new compounds were sufficiently effective as residual insecticides to warrant further testing. Nine compounds caused 90% to 100% control of bed bugs for a period of at least 24 weeks in laboratory tests.

Four colonies of insecticide resistant (DDT and malathion) bed bugs are being maintained as a source of insects for testing promising new insecticides for cross-resistance and evaluating and developing standardized techniques for assaying resistance levels in populations of bed bugs. Recent tests showed standard papers in the bed bug resistance test kit of the World Health Organization treated with DDT or dieldrin to retain their effectiveness for at least 48 months.

C. Biological Control

1. Mosquitoes. At Lake Charles, La., research on mosquito pathogens as potential biological control agents has been increased. Microsporidian, bacterial, fungal, viral, and/or nematode parasites have been found in 35

species of mosquitoes in Louisiana. Microsporidian parasites have been found in about 17 species of mosquitoes including infections of Thelohania, Stempellia, Plistophora, and Nosema. Microsporidian parasites have been grouped according to host parasite relationships described by Kellen and coworkers. Transovarial transmission has been demonstrated. Most infections found in field populations of mosquito larvae tend to be of a low order (around 1%). Means and methods of increasing infectivity need to be developed. Corothrella appendiculata, a very small chaoborid which inhabits tree holes and artificial containers was observed to be infected with an unknown species of microsporidia. A colony of Culex salinarius infected with Thelohania has been established in the laboratory for experimental purposes. Since only adult females survive from infected egg rafts it is necessary to add males from a non-infected colony to keep the infected colony going.

A species of Spirillum (a bacteria) which invades the hemocoel has been isolated from 12 species of mosquitoes. Infection rates are generally low in field populations. For example, approximately one percent of Culex p. quinquefasciatus collected from a roadside ditch polluted by septic tank effluent were infected.

Coelomomyces infections have been identified in eight species of mosquitoes. An infected colony of Culiseta inornata has been established for laboratory studies and a naturally infested pothole in the field is being studied.

A virus has been identified in Aedes sollicitans, A. taeniorhynchus and A. vexens. A colony of A. taeniorhynchus infected with this virus is being maintained for basic studies of infectivity within and between different species of mosquitoes. Nematodes and internal ciliates have been identified in several species of mosquitoes.

Colonies of Culex p. quinquefasciatus, C. salinarius, Aedes triseriatus, and Culiseta inornata are well established in the laboratoryfor basic research on biology and pathogens.

At Corvallis, Oreg., research was initiated on microsporidian infections of mosquitoes found in log ponds. Infections were found in Culex pipiens, C. peus, and Culiseta incidens. A technique for determining per os transmission has been developed; however, methods of transmission other than transovarian have not been demonstrated.

At Gainesville, Fla., research on pathogens of mosquitoes has been increased. Microsporidial parasites have been found in Culex salinarius, C. restuans, Anopheles quadrimaculatus, and A. crucians. It has been impossible to infect healthy larvae with these parasites so research is designed to elucidate life cycle and biology of the parasites. Fungi of the genus Coelomomyces and Achlya have been isolated from several species of mosquitoes as well as several species of bacteria. A microsporidian has been found infecting a species of chironomid.

Under contract, the Fresno Laboratory of the California State Public Health Department through the University of California at Berkeley is researching pathogens as biological control agents. Various species of bacteria--Bacillus cereus, B. thuringiensis, B. sphaericus, Aeromonas hydrophila, Pseudomonas, sp., Flavobacterium sp., and Achromobacter sp.; fungi--Spicaria farinosa and Coelomomyces psorophorae; microsporia; and viruses have been identified as pathogens in western species of mosquitoes. Host-parasites relationships and methods of infectivity are now under study.

Under contract funds with the McNeese State College at Lake Charles, La., research is being conducted on predators of mosquito larvae. Studies are in progress on insects and fish as predators of mosquito larvae and have included Mollienesia latipinna and Cyprinodon variegatus and species of the family Corixidae. Some observations indicate corixids may lay their eggs on crawfish. This is of special interest in relation to Psorophora confinnis in Louisiana rice fields where many owners are alternating between rice and crawfish crops.

2. House Flies. A grant with the University of California has been negotiated to study the effect of predacious mites on populations of flies breeding in manure. Progress to date has been limited to identifying mite species and determining density of mites in various fly breeding sites.

A PL-480 project in Korea is making excellent progress in identifying species of beetles present in dung and defining their role in the dispersion of dung with related effects on fly breeding. Progress to date is mainly in determining species, their distribution, biology, and life cycles.

3. Imported Fire Ant. Under PL- 480 support, research was continued on parasites of the imported fire ant in Uruguay. The distribution of imported fire ant colonies has been studied and recorded. Data has been collected on various forms of arthropods found in ant mounds. Of particular interest have been studies on an ant, Labauchena daguerri, which parasitises the imported fire ant. Laboratory and field studies have shown that L. daguerri is apparently dependent upon the imported fire ant for survival and weakens imported fire ant colonies in the field.

D. Insect Sterility and Other New Approaches to Control

1. Mosquitoes. At Gainesville, Fla., research was continued on the evaluation of the sterility principle for control of mosquitoes. The effect of varying dosages of gamma radiation on fecundity and fertility of Aedes aegypti treated in the pupal stage was determined. Females were completely sterilized at 3,000 r, while males were completely sterilized at 10,000 r. Male Culex p. quinquefasciatus were almost completely sterilized at doses of 7,000 to 12,000 r. Males irradiated at 8,500 and 10,000 r were less competitive than untreated males in mating. Males of C. p. quinquefasciatus were effectively sterilized by dusting with apholate powder. The sterility induced was perminent and the sterilized males were competetive with untreated males in mating untreated females.

Two sterile male release studies were made with a semi-isolated population of Anopheles quadrimaculatus. Males were sterilized by exposure to residual deposit of tepa. Although some sterility was induced in the natural population (up to 42%), no reduction in the numbers in the population occurred. A bait of apholate was placed in natural resting stations of A. quadrimaculatus in this same area in another experiment. Although a high degree of sterility occurred in mosquitoes feeding on the bait, there was little sterility in the natural population and no reduction in its size.

The colony of Aedes aegypti which developed resistance to apholate through laboratory selection is being maintained. Previously reported resistance was confirmed and the level has continued to increase. Tests indicated that this apholate-resistant colony was not resistant to tepa. Studies have been initiated to determine the mechanism involved in resistance to apholate.

2. House Flies. Research was continued at Gainesville, Fla., on the development of sterilization techniques for house fly control. Six hundred and thirteen chemicals were screened for chemosterilant activity and 67 of these caused complete sterility in adults. Twenty-five of the chemicals also caused complete sterility in males. Tests were conducted to determine whether chemicals causing sterility in house flies affected sperm motility. All of the chemosterilants found in screening tests to date were evaluated and none had any effect on sperm motility. One hundred and seventy two chemosterilants were evaluated for sterilizing effects when they were applied to house fly rearing medium containing third instar larvae. Eleven of these chemicals caused complete sterility by preventing oviposition or egg hatch. House fly pupae immersed in solution of hempa or 2,4-diamino-6-(2-furyl)-s-triazine for periods of 5 to 30 minutes were sterilized. However, the sterilizing dose was probably acquired mostly through adult contact with treated pupal cases during emergence since washing pupae after treatment reduced sterility to low levels (35% to 46%). Hempa caused a reduction in cholinesterase activity in in vivo and in vitro tests with house flies.

Tests were conducted on Grand Turk Island in the Bahamas to evaluate the effectiveness of an integrated program for the control of house flies. The procedure consisted of making insecticide and chemosterilant applications to larval breeding sites as well as releasing adult flies that had been sterilized by gamma irradiation. High population reductions were achieved through the application of larvicides and chemosterilant baits and there was a high degree of sterility in the population of house flies remaining on the island. The release of sterile insects appeared to increase the level of sterility in the natural population but not sufficiently to eradicate the population. Either the number of sterile insects released was too low or their behavior and dispersion was abnormal. Research has been undertaken to study dispersion of released house flies on the island and the density of the natural population existing on the island. House fly populations were reduced in numbers by the integrated control scheme to approximately 1,000,000.

At Corvallis, Oreg., research was undertaken on methods of sterilizing the little house fly. Tepa, metepa, apholate, hempa, and hemel were evaluated in the laboratory as chemosterilants. Residual treatments of tepa (0.1-1.0 mg/ft^2) sterilized these flies. Metepa and hempa were somewhat less effective, and apholate failed to sterilize at 50 mg/ft^2. When fed to adult flies, tepa was again the most effective, producing a high degree of sterility at concentrations of 0.01 to 0.1% in adult food. The other materials were about equally effective. In experiments on mating competitiveness, males treated with hempa and metepa competed well with untreated males. There was no great margin between dosages causing complete sterility, especially of females, and those causing some mortality. Pupae were unaffected when dipped in 0.05 to 5% ethanol solutions of metepa.

3. Cockroaches. At Gainesville, Fla., basic studies were undertaken on the effects of chemosterilants on German cockroaches and on embryological development in German cockroach eggs. Studies on the development of eggs from unmated females, showed, in adition to normal embryonic development, various degrees of deformity. A fully-developed embryo was removed from an ootheca of an unmated female and it eventually became an adult female but failed to mate or reproduce.

Male German cockroaches were completely sterilized by injections of 3.0 ug of tepa. Eggs from normal females mated to these sterile males showed some normal embryonic development and various degrees of deformity. The degrees of development or deformity that occurred were within the range of those observed in eggs from unmated females. Adult females were injected with doses of 3, 4, 5, 6, 8, and 10 ug of tepa and mated with untreated females Some hatch occurred at all doses except 10 ug which also caused high mortalities of the treated females.

4. Tsetse Flies. Research was continued in Salsibury, Rhodesia, in cooperation with the Agricultural Research Council of Central Africa on the feasibility of the sterile male technique for tsetse flies. Following the successful development of effective methods of chemosterilizing 2 species of tsetse, Glossina morsitans and G. pallidipes, field tests demonstrated that sterile males are competitive with normal males in small cage tests and that sterile males disperse and survive as well as normal males under natural field conditions. An island has been selected for a small field experiment on the release of sterile males. Approximately 9-months data has been accumulated on population levels, dynamics, survival, and birth rates to serve as a basis for designing a release experiment. Progress had been made in developing techniques for establishing self-sustaining colonies of tsetse in the laboratory or in cages in field environments; however, a productive self-sustaining colony for use in a release experiment or program has not been developed to date.

E. Attractants and Repellents

1. Mosquitoes. At Gainesville, Fla., the screening of new compounds and competitive testing of promising compounds as personal-use repellents has been continued. The current Aedes aegypti eradication program has led to research on the suitability of other species of mosquitoes as possible replacements for Aedes aegypti in laboratory test procedures. Tests were run to compare four single repellents and one repellent mixture as skin applications with Aedes triseriatus and A. aegypti. All repellents--deet, ethyl hexanediol, dimethyl phthalate, dimethyl carbate, and M-2020--with the exception of dimethyl carbate, gave a high degree of protection from bites of A. triseriatus; however, only ethyl hexanediol, and deet were equally effective against both species. Two of six compounds prepared by the University of Tennessee College of Pharmacy showed promise as repellents. Deet and a Russian repellent were compared as skin repellents. Deet gave a longer protection time in laboratory tests with A. aegypti than the Russian repellent. A lanolin formulation of deet gave slightly longer protection time against A. aegypti than deet in ethanol solution.

With the cooperation of members of the SEATO Laboratory in Thailand, six individual repellents and three repellent mixtures were evaluated against mosquitoes under practical field conditions. All the repellents were highly effective.

Research on the development of improved repellents for clothing treatments for protection against mosquitoes was continued. Only one of 61 new compounds tested showed sufficient promise for further testing. Research is also underway to find space repellents for use against mosquitoes. Of 662 chemicals evaluated in the laboratory, 99 prevented mosquitoes for 6 days or more from penetrating 4-mesh netting treated with the chemicals. Fourteen chemicals prevented penetration for 80 days or more. Field tests were conducted with promising materials as space repellents. N,N-dibutyl-o-ethoxybenzamide, o-ethoxy-N,N-diethylbenzamide and 2-methylbutyl-m-chlorocarbanilate at 3.5 g/ft^2 on netting reduced mosquito penetration by at least 80% for 36 to 84 days.

At Gainesville, Florida, studies were continued on the factors affecting mosquito response to human hosts. It was shown that attractant response of Aedes aegypti to chloroform and human hosts was greatly reduced following removal of 6 distal segments from female antennae. Biting rates of this species declined rapidly after 4 distal antennae segments were removed.

A new olfactometer was developed to study mosquito response to attractants. About 16% of the Aedes aegypti mosquitoes studied exhibited an anemotactic response. The addition of CO_2 increased the anemotactic response to about 48%. A human arm alone stimulated positive anemotaxis in approximately 35% of the mosquitoes. The behavioral response to a repellent treated arm was

also studied. The response of mosquitoes to CO_2 in the presence of the repellent-treated arm indicated that two entirely different olfactory receptors could be involved in attraction to CO_2 and to an arm. Studies were designed to measure the response of mosquitoes to convected or radiated heat. Some attraction was observed but further studies are needed.

At Corvallis, Oreg., screening tests have been undertaken to find attractants for Culex quinquefasciatus. Of 33 chemicals tested for attractancy in an olfactometer, none showed any significant attraction for this species. Movement of C. quinquefasciatus females into olfactometer traps indicated attraction of females to males and to a lesser degree to females suggesting the existence of pheromones in this species. Tests with benzene extracts of this mosquito further indicated the existence of pheromones. Some attraction of females to males with C. tarsalis has also been observed. Extracts of log pond waters and cold trapped odors from the same waters were attractive to gravid females of C. quinquefasciatus and C. tarsalis.

2. House Flies. At Gainesville, Fla., a method was developed to screen various chemicals as attractants (olfactory stimulation) for house flies. Various tests showed that edamin could be used as a standard, although its ability to attract flies is minimal. Of 113 chemicals that have been screened none have shown a high degree of attractancy. Certain chemicals produced an anomalous avoidance or repellency response.

Tests were run on certain chemosterilants to determine if they were repellent to house flies. Apholate was somewhat repellent and metepa and hempa highly repellent. Hempa did not attract or repel house flies by olfaction so repellency may be tactile or gustatory.

Other tests showed that the age or condition of ovaries influenced the responsiveness of female house flies to ammonium hydroxide. Response differed with differing concentrations and a concentration of 1.0 N ammonium hydroxide was preferred over all others.

Research was continued at Corvallis, Oreg., on the sex pheromone reported earlier in the house fly. Considerable emphasis has been given to cooperative work with chemists of the Pesticide Chemicals Research Branch attempting to isolate active components or fractions of the pheromone. Isolation has not been accomplished to date. It was confirmed that the presence of the pheromone in female flies was age related. No house fly sex pheromone-like activity was present in benzene extracts of female or male black blow flies, female false stable flies, female or male stable flies, or female or male little house flies. To date clear demonstrations of activity have been observed only with extracts of female and male horn flies, and female house flies.

Approximately 90 miscellaneous compounds were screened as attractants for the little house fly in field tests near natural populations. None of the

compounds showed an attraction of these flies or other muscid, calliphorid, and sarcophagid flies in the area.

3. Cockroaches. In laboratory tests, 10 materials were evaluated as possible attractants for male and female German cockroaches. None of them exhibited appreciable attractiveness.

4. Mites, Ticks, and Fleas. Research was continued at Gainesville, Fla., on the development of clothing treatments as repellents for ticks and acaricides for mites. In field tests in cooperation with the Navy Medical Field Research Laboratory at Camp LeJeune, North Carolina, N,N-dibutyl-p-toluamide and M-1960 applied to uniforms at the rate of 2 gm/ft^2 and 3.6 gm/ft^2 gave excellent protection from ticks after 48 hours of wear. Deet, N,N-dibutyl-m-toluamide, and repellent mixtures M-2126, M-2127, and M-2099 were slightly less effective, giving protection up to 40 hours of wear. N,N-dibutyl-p-toluamide, alone and in mixture with M-2127, produced an unpleasant odor in the treated uniforms.

In the development of chigger repellents or toxicants, screening tests were completed on 14 chemical materials compared to the standard, benzyl benzoate. The two most effective compounds were lindane, which withstood an average of 9.5 washes and Shell SD-8530 which withstood 4.0 washes. Benzyl benzoate survived an average of 1.5 washes.

Knockdown tests were conducted against chiggers to determine the effectiveness of various dosages of four compounds after washing. Lindane was effective after three washes at 0.25 gm/ft^2, and after one wash at 0.05 gm/ft^2. Shell SD-8530 was effective after three washes at 2.0 gm/ft^2, and after one wash at 0.25 gm/ft^2. Carbaryl was effective after two washes at 2.0, 1.0, and 0.5 gm/ft^2, and after one wash at 0.1 gm/ft^2.

Various concentrations of three chemicals and the standard, benzyl benzoate were compared in the field as sleeve treatments against chiggers. The most effective materials were lindane and carbaryl which gave 100% protections after 3 washes when applied at 0.125 gm/ft^2, and 1 gm/ft^2, respectively.

5. Leeches. At Gainesville, Fla., repellent tests were continued against bloodsucking leeches, Macrobdella decore, and a Korean leech of the genus Hirudo. New repellents that were highly effective and survived rinsing effects of water were N,N-dibutyl-o-toluamide and lanolin, MGK-264 and lanolin, 2-butoxyethyl octyl sulfoxide and lanolin, N,N-dibutyl-p-toluamide and lanolin, and N,N-diisobutyl-m-toluamide alone. Deet, a material shown previously to be an effective leech repellent, combined with lanolin U.S.P. was as effective, or more so, as a leech repellent than deet combined with acetylated lanolin.

In August 1965 a series of tests was conducted in Rayong, Thailand, in cooperation with military entomologists to evaluate three individual

repellents (deet and two dibutyltoluamides) and three repellent mixtures containing deet as repellents for terrestial leeches of the genus Haemadispa. Test subjects treated their feet and legs to the knee, waded through water, and then exposed themselves to leeches in nearby vegetation. Based on the amount of wading withstood, deet alone, N,N-dibutyl-m-toluamide alone, a mixture of deet and lanolin, and a mixture of deet and N,N-dibutyl-p-toluamide were about equal in effectiveness. N,N-dibutyl-p-toluamide alone and deet plus N,N-dibutyl-m-toluamide were more effective than the other four repellents.

PUBLICATIONS --- USDA AND COOPERATIVE PROGRAMS

Basic Biology, Physiology, and Nutrition

Bigley, W. S. 1966. The inhibition of cholinesterase and aliesterase in parathion and paraoxon poisoning in the house fly. J. Econ. Entomol. 59: 60-65.

Chapman, H. C. and D. B. Woodard. 1965. Blood feeding and oviposition of some floodwater mosquitoes in Louisiana laboratory studies. Mosq. News 25: 259-62.

Fye, R. L. and G. C. LaBrecque. 1966. Sexual acceptability of laboratory strains of male house flies in competition with wild strains. J. Econ. Entomol. 59: 538-40.

Hoyer, R. F. 1966. Some new mutants of the house fly (Musca domestica L.) with notations of related phenomena. J. Econ. Entomol. 59: 133-37.

Hoyer, R. F., F. W. Plapp, Jr., and R. D. Orchard. 1965. Linkage relationships of several insecticide resistance factors in the house fly. Entomol. Exp. Appl. 8: 65-73.

Mayer, M. S. 1966. The decline in the male/female sex ratio of Aedes aegypti (L.) during hatching. Mosq. News 26: 82-84.

Murvosh, C. M. and C. W. Thaggard. 1966. Ecological studies on the house fly. Ann. Entomol. Soc. Amer. 59: 533-47.

Roberts, R. H. 1966. A technique for rearing the immature stages of Tabanidae (Diptera). Entomol. News Vol. LXXVII, No. 3.

Schwartz, P. H., Jr. 1965. Some observations on the reproduction of the eye gnat, Hippelates pusio. J. Med. Entomol. 2: 141-44.

Schwartz, P. H., Jr. and R. B. Turner. 1966. A dietary study of the adult eye gnat, Hippelates pusio. Ann. Entomol. Soc. Amer. 59: 277-80.

Smith, C. N. 1965. Need for standard and other strains of Aedes aegypti for research. World Health Organ./Vector Control/190.65: 117-20.

Woodard, D. B. and H. C. Chapman. 1965. Blood volumes ingested by various pest mosquitoes. Mosq. News 25: 490-91.

Conventional Insecticide Control Methods

Brady, U. E. 1966. A continuous exposure technique for determination of insecticide resistance in house flies. J. Econ. Entomol. 59: 764-65.

Burden, G. S. and Smittle, B. J. 1965. Household insects and their control. Consumers All. U.S.D.A. Yearbook Agric.: 187-93.

Gahan, J. B., B. M. Glancey, and C. S. Lofgren. 1965. New larvicides that show promise against mosquitoes. Proc. 52nd Ann. Mtg. New Jersey Mosquito Extermination Assoc., Atlantic City: 156-59.

Gahan, J. B., H. G. Wilson, and C. N. Smith. 1965. Additional studies with new insecticides as residual sprays in buildings naturally infested with Anopheles quadrimaculatus. Bull. World Health Organ. 32: 169-73.

Gahan, J. B., W. W. Young, N. E. Pennington, and G. C. LaBrecque. 1965. Thermal aerosol and larvicide tests with new insecticides to control two species of Culex mosquitoes on Okinawa. Mosq. News 25: 165-69.

Glancey, B. M., C. S. Lofgren, J. Salmela, and A. N. Davis. 1965. Low volume aerial spraying of malathion for control of adult salt-marsh mosquitoes. Mosq. News 25: 135-37.

Gjullin, C. M. and G. W. Eddy. 1965. Low volume airplane sprays for the control of mosquitoe larvae. Mosq. News 25: 442-47.

LaBrecque, G. C., M. C. Evers, and D. W. Meifert. 1966. Control of house flies in outdoor privies with larvicides. J. Econ. Entomol. 59: 245.

Lewis, L. F., D. M. Christenson, and G. W. Eddy. 1965. Control of mosquito larvae in Willamette Valley, Oregon, log ponds. Mosq. News 25: 400-402.

Lofgren, C. S., N. Pennington, and W. Young. 1966. Evaluation of insecticides against two species of Culex mosquitoes on Okinawa. Mosq. News 26: 52-59.

McDuffie, W. C., B. M. Glancey, J. B. Gahan, and L. F. Lewis. 1965. Review of recent research on mosquitoes by the Entomology Research Division, U.S.D.A. Proc. and Papers 33rd Ann. Conf. Calif. Mosquito Assoc. Inc. 33: 15-19.

McDuffie, W. C. and D. E. Weidhaas. 1965. The future outlook for new and improved materials and methods for mosquito control. Mosq. News 25: 85-91.

Morgan, P. B., G. C. LaBrecque, and H. G. Wilson. 1966. Tests with larvicides for the control of house flies (Musca domestica L.) in poultry houses. The Florida Entomol. 49: 91-93.

Mount, G. A., J. B. Gahan, and C. S. Lofgren. 1965. Evaluation of insecticides in the laboratory against adult and larval stable flies. J. Econ. Entomol. 58: 685-87.

Hoyer, R. F. and F. W. Plapp, Jr. 1966. A gross genetic analysis of two DDT-resistant house fly strains. J. Econ. Entomol. 59: 495-501.

Plapp, F. W., Jr., G. A. Chapman, and J. W. Morgan. 1965. DDT resistance in Culex tarsalis Coquillett: cross resistance to related compounds and metabolic fate of a C^{14} labeled DDT analog. J. Econ. Entomol. 58: 1064-69.

Plapp, F. W., Jr., R. D. Orchard, and J. W. Morgan. 1965. Analogs of parathion and malathion as substitute insecticides for the control of resistant house flies and the mosquito, Culex tarsalis. J. Econ. Entomol. 58: 953-56.

Plapp, F. W., Jr., and Homer H. C. Tong. 1966. Synergism of malathion and parathion against resistant insects: phosphorus esters with synergistic properties. J. Econ. Entomol. 59: 11-15.

Smith, C. N. 1964. Possible future countermeasures for resistance. Proc. 7th Intern. Cong. on Tropical Med. & Malaria: 5: 12-13.
Smith, C. N. 1964. Research needs and priorities: chemical control of Culex fatigans. World Health Organ./Vector Control/125.65 (Paper 8.8) 212-15.
Smith, C. N. and J. B. Gahan. 1964. Control of Anopheles quadrimaculatus Say with residual applications of carbamate insecticides. Insecticides and Toxicol. Sect. 8. Proc. of XII Intern. Cong. of Entomol.
Smittle, B. J., and G. S. Burden. 1965. Dichlorvos as a vapor toxicant for the control of cockroaches, bed bugs, and fleas. Pest Control 33: 26-32.

Insect Parasites, Predators, and Pathogens

Cole, M. M. 1965. Biological control of ticks by use of hymenopterous parasites. World Health Organ/EBL Series/43.65.
Gingrich, R. E. 1965. Thelophania tabani n. sp., a Microsporidian from larvae of the black horse fly Tabanus atratus Fabricius. J. Invert. Patho. 7: 236-40.

Insect Sterility and Other New Approaches to Control

Eddy, G. W., A. R. Roth, and L. R. Abrahamsen. 1965. Sterilant effect of some materials on Aedes aegypti (L.) feeding on treated mice. Mosq. News 25: 169-71.
Fye, R. L., G. C. LaBrecque, and H. K. Gouck. 1966. Screening tests of chemicals for sterilization of adult house flies. J. Econ. Entomol. 59: 485-87.
Glancey, B. M. 1965. Hempa as a chemosterilant for the yellow-fever mosquito (Aedes aegypti (L.)) (Diptera: Culicidae). Mosq. News 25: 392-96.
LaBrecque, G. C. 1964. Chemosterilants for the control of insects. Proc. of XII Intern. Congr. of Entomol., London. Section 8 Insecticides and Toxicol. 515-16.
LaBrecque, G. C. 1964. Advances in research on the control or eradication of insects with chemosterilants. Series No. 44, 79 pp. International Atomic Energy Agency Panel on Advances in Insect Control by the Sterile Male Technique.
LaBrecque, G. C., P. B. Morgan, D. W. Meifert, and R. L. Fye. 1966. Effectiveness of hempa as a house fly chemosterilant. J. Med. Entomol. 3: 40-43.
McDuffie, W. C. and D. E. Weidhaas. 1965. The future outlook for new and improved materials and methods for mosquito control. Mosq. News 25: 85-91.
Schwartz, P. H., Jr. 1965. The effects of apholate, metepa, and tepa on the reproductive tissues of Hippelates pusio Loew. J. Insect Pathol. 7: 148-51
Smith, C. N. 1966. Research needs and priorities: Genetic control, including sterile male technique. World Health Organ./Vector Control/190.65 Seminar on the Ecology, Biology, Control and Eradication of Aedes aegypti 255-58.

Smith, C. N. 1966. Possible use of the sterile male technique for Aedes aegypti control. World Health Organ./Vector Control/190.65 Seminar on the Ecology, Biology, Control and Eradication of Aedes aegypti 213-18.
Weidhaas, D. E. 1965. Research on radioisotopes and radiation in insect control related to the development of the sterile male technique. 5th Inter-Amer. Symp. Peaceful Appl. Nucl. Energy, Valparaiso, Chile. 189-95.

Attractants and Repellents

Gilbert, I. H. 1966. Evaluation and use of mosquito repellents. J. Amer. Med. Assoc. 196: 253-55.
Gjullin, C. M., J. O. Johnsen, and F. W. Plapp, Jr. 1965. The effect of odors released by various waters on the oviposition sites selected by two species of Culex. Mosq. News 25: 268-71.
Gouck, H. K. 1966. Protection from ticks, fleas, chiggers, and leeches. Archeol. Dermatol. 93: 112-13.
Smith, C. N. 1964. Insect repellents--their usefulness and future development. Insecticides and Toxicol. 507-509.
Smith, C. N. 1966. Personal protection from blood-sucking arthropods--an introduction to the symposium. J. Amer. Med. Assoc. 196: 236-39.

PUBLICATIONS--STATE EXPERIMENT STATIONS AND COOPERATIVE PROGRAMS

Basic Biology, Physiology, and Nutrition

Anderson, J. F. and W. R. Horsfall. 1965. Thermal stress and anomalous development of mosquitoes (Diptera: Culicidae). V. Effect of temperature on embryogeny of Aedes stimulans. J. Exp. Zool. 158: 211-22. (Conn.)
Hayes, Kirby L. 1965. Longevity, fecundity, and food intake of adult Triatoma sanguisuga (Leconte) (Hemiptera: Triatominae). Med. Ent., 2: 200-202. (Ala.)
Hitchcock, S. W. 1965. The seasonal fluctuation of limnetic Chaoborus punctipennis and its role as a pest in drinking water. J. Econ. Entomol. 58: 902-904. (Conn.)
Jones, J. C. and K. W. Ludlam. 1965. Aedes aegypti with four spermathecae. Mosq. News 25: 67. (Md.)
Jones, J. C. and R. E. Wheeler. 1965. Studies on spermathecal filling in Aedes aegypti (Linnaeus). I. Description. Biol. Bull. 129(1):134-50 (Md)
Knight, K. L. 1965. Some physical and chemical characteristics of coastal soils underlying mosquito breeding areas. Mosq. News 25: 154-59. (Iowa)
Pimentel, D., E. H. Feinberg, P. W. Wood and J. T. Hayes. 1965. Selection, spatial distribution, and the coexistence of competing fly species. Amer. Natur. 99: 97-109. (N.Y.)
Ross, M. H. and D. G. Cochran. 1965. A preliminary report on genetic variability in the German cockroach, Blattella germanica. Ann. Entomol. Soc. Amer. 58: 368-75. (Va.)
Wagner, R. E., W. Ebeling, and D. A. Reierson. 1965. Controlled environment chambers for the biological laboratory. J. Econ. Entomol. 58: 236-40. (Calif.)

Wright, C. G. 1965. Identification and occurrence of cockroaches in dwellings and business establishments in North Carolina. J. Econ. Entomol. 58: 1032-33. (N.C.)

Conventional Insecticide Control Methods

Ebeling, W. and R. E. Wagner, 1965. Influence of humidity on the volatilization of insecticidal residues as affected by formulation and substrate. J. Econ. Entomol. 58: 240-45. (Calif.)

Georghiou, G. P., and Frances E. Gidden. 1965. Contact toxicity of insecticide deposits on filter paper to adult mosquitoes. Mosq. News 25: 204-8. (Calif.)

Grayson, J. M. 1965. Resistance to three organophosphorus insecticides in strains of the German cockroach from Texas. J. Econ. Entomol. 58: 956-58. (Va.)

Knapp, F. W. and W. W. Roberts. 1965. Low-volume aerial application of technical malathion for adult mosquito control. Mosq. News 25: 46-7. (Ky.)

Mulla, M. S. 1965. Field evaluation of organophosphate insecticides as soil treatments for the control of Hippelates gnats. J. Econ. Entomol. 58: 654-58. (Calif.)

Pence, R. J. and M. S. Viray. 1965. Tests with Kepone and Mirex for the control of carpet beetle larvae in fabric. J. Econ. Entomol. 58: 916-20. (Calif.)

van den Heuvel, M. J. and D. G. Cochran. 1965. Cross resistance to organophosphorus compounds in malathion- and diazinon-resistant strains of Blattella germanica. J. Econ. Entomol. 58: 872-74. (Va.)

Insect Parasites, Predators, and Pathogens

Bay, E. C. and L. D. Anderson. 1965. Chironomid control by carp and goldfish. Mosq. News 25: 310-16. (Calif.)

AREA NO. 15. BEES AND OTHER POLLINATING INSECTS

Problem. Bees contribute to the production of crops occupying at least 5 million acres. Their pollinating activities perform a necessary service in the production of fruit crops, such as, deciduous fruits, berries, cucumbers, melons, and also for vegetable and legume seed crops which are required for the planting of many hundreds of thousands of acres. This pollinating service is performed incidental to foraging for foods for their own use (nectar and pollen). Honey bees are the most important of all the insect pollinators.

Beekeeping is practiced in all 50 states. This distributes pollinators throughout all the cultivated areas. It makes available a manageable supply of pollinators for use where and when they are required. A self-supporting pollination service for agricultural crops is provided through the production and sale of honey and beeswax. To maintain an adequate level of pollinators, however, beekeeping must be kept in a profitable condition.

A problem of major significance is the increasing use of pesticides, many of which are hazardous to bees or destroy important pollen and nectar sources. There is need for more knowledge of the management of bee colonies; breeding of improved hybrid bees; physiology and behavior of queens, drones, and workers; and the various diseases and pests of the honey bee and means for their control. There is also need to study the many facets of the complex pollination problem to integrate effectively populations of honey bees and other pollinating insects with crop needs and practices. More knowledge should be obtained about wild insect pollinators and their management. It is also essential to study the effects of farm practices, such as the use of different pesticides, changes in crops, soil management, and harvesting, on the economy of the beekeeping industry and the survival of pollinating insects, and to develop procedures to minimize losses from such practices. Information is needed on nectar and pollen plants for use in conservation program efforts to provide bee forage areas in wastelands, watersheds, and roadsides. The nutrition of bees and the nutritive value of different pollens to bees require intensive investigation together with basic nutrition studies for development of pollen substitutes.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving apiculturists, geneticists, microbiologists, physiologists, and entomologists engaged in basic studies and in research concerned with the application of known principles to the solution of problems in crop pollination by insects for the farmer and problems that affect the beekeeper. Bee breeding investigations at Baton Rouge, La., are cooperative with the State Experiment Stations of Louisiana, Ohio, California, and Wisconsin, and the Ontario Agricultural College, Guelph, Ontario, Canada. Bee management investigations at Madison, Wis., are

cooperative with the Wisconsin and Arizona Experiment Stations, the Department of Apiculture at Ontario Agricultural College, Canada, and the Agricultural Engineering Research Division. Investigations on bee diseases are carried on at Beltsville, Md., and Laramie, Wyo., in cooperation with the Louisiana, Wisconsin, and Wyoming Experiment Stations. Honey bee pollination investigations at Tucson, Ariz., are carried on in cooperation with the Experiment Stations of Arizona, California, Louisiana, Utah, and Wisconsin, and the Agricultural Engineering Research and Plant Pest Control Divisions of ARS. Wild bee pollination investigations at Logan, Utah, are conducted in cooperation with the Experiment Stations of Arizona, Utah, Louisiana, Wyoming, Idaho, Oregon, Washington, the Crops and Agricultural Engineering Research Divisions of ARS, and private beekeepers and farmers.

The Federal scientific effort devoted to apiculture research totals 12.0 scientific man-years. Of this number 3.0 is devoted to biology and breeding for improvement of the honey bee; 1.0 to management for improvement in productivity of honey bees; 2.4 to etiology of bee diseases and development of control methods for diseases and pests; 3.6 to behavior and utilization of honey bees in the pollination of agricultural and other economic crops; 1.0 to biology and utilization of insects other than honey bees in the pollination of agricultural crops; and 1.0 to effect of pesticides and farm practices on honey bees and other pollinating insects.

In addition Federal support of research is provided under a grant to Ohio State University for 0.5 man-year for bee disease research, specifically Nosema; a grant to Utah State University for 0.35 man-year for bee gland research; and a grant to University of Illinois for 0.3 man-year for bee pheromone studies.

Apiculture research conducted under P.L. 480 grants total 18.75 man-years. Bee breeding research is being conducted at the Central Apicultural College, Warsaw, Poland (5.5 man-years), at the Research Institute of Pomology, Skierniewice, Poland (2.5 man-years), and at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Sao Paulo, Brazil (3.0 man-years). Bee disease research is underway with the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (3.0 man-years) and with the Istituto Nazionale di Apicoltura, Bologna, Italy (0.75 man-year). Wild bee pollination research is being conducted with the Faculty of Agriculture, Department of Agricultural Zoology, University of Cairo, Egypt (1.5 man-years) and with the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (2.5 man-years).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 12.0 professional man-years is devoted to this area of research.

PROGRESS - USDA AND COOPERATIVE PROGRAM

A. Biology and Breeding for Improvement of the Honey Bee

1. Superior Hybrid Bee for Honey Production. At Madison, Wis., two superior hybrid strains averaged honey yields of 328 and 302 pounds per colony in 1965. In 1964, these same hybrid strains averaged 317 and 303 pounds respectively. These two superior hybrid stocks will be made available to industry as soon as a stock center has been established.

2. Holding Colonies for Queen Storage. Queens removed from small colonies at Madison during the winter period and placed in special holding colony units successfully survived the winter. Sixty-seven such queens were held until mid-May in the special holding colonies in which they were overwintered. Future plans are to hold breeding stock queens in such queen bank colonies at Madison.

3. Alfalfa Pollen Collecting Strains. Studies were continued on the alfalfa pollen collecting project designed to produce high and low alfalfa pollen collecting lines of bees with the breeding work being done at Baton Rouge, La., and the testing work done at Logan, Utah. The 1965 tests showed that by continuous selections the two lines are moving farther apart. The high line averaged 66.4 percent alfalfa pollen collectors and the low line averaged 7.5 percent. During 1965, a hybrid between these two lines was tested for the first time. It averaged 34.1 percent, indicating that additive gene effects account for most of the differences between the lines. Queens have been produced for 1966 tests at Logan, Utah, and Madison, Wis. The genetics of this characteristic will be further studied in 1966 in backcrosses to hybrids. Studies of inbred bees of these lines fail to show significant morphological differences between the two lines.

4. Cranberry Pollen Collecting Bees. During a cooperative study between the Beltsville, Md., laboratory and commercial and state workers in New Jersey, a colony of bees was observed collecting over 95% cranberry pollen when all other colonies in the area were collecting about 20% cranberry pollen. This colony and queen have been transferred to Beltsville, where queens are being produced for transmittal to Baton Rouge for breeding and maintenance. If this character should prove heritable, this strain may be of great importance for specific pollination of cranberries.

5. Diploid Drones. Bee breeding research under P.L. 480 funds at the Central Apicultural College, Warsaw, Poland, (E21-ENT-7), which produced the extremely significant contribution to bee genetics by demonstrating that queen bees do produce diploid drone eggs, has been extended for another five years. The objective of these studies will be to make a thorough study of diploid drones and to develop practical methods of rearing these drones to maturity. In addition to the basic information developed here, considerable impact on practical beekeeping may result. The use of diploid drones would

greatly widen the scope of bee genetics research and enhance the chances of obtaining the types of bees needed for greater honey and wax production and improved pollination.

6. Mating Stations. Research on bee breeding is also being conducted at the Research Institute of Pomology, Skierniewice, Poland, (E21-ENT-10). These studies relate to the distance of mating flights of queen and drones and the necessary isolation of mating stations for preventing mismatings. Information developed under this project should be helpful for queen producers to produce commercially a better queen at a reasonable cost to the beekeeper and without resorting to artificial insemination.

7. Wild Bees. Research on bee breeding is also being conducted at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Sao Paulo, Brazil (S3-ENT-1). Under this project, diploid drones or sperms have been produced by the injection of colchicine in saline; a positive correlation has been shown between humidity and total length of Malpighian tubules found in two species of Trigona and three species of Melipona; a detailed observation and description of the mating procedure of Bombus (fervidobombus) atratus Franklin has been made and drawings of the nest architecture and behavior of some of the stingless bee species have been added to the literature.

B. Management for Improvement in Productivity of Honey Bees

1. Increased Size of Shipping Packages of Bees. In a test to determine the feasibility of using large shipping packages, 22 pounds of bees were placed in a cage 8x13x30 inches on January 4 at Madison, Wis., provided with syrup and 4 caged queens suspended around the syrup can. At the time of installation, the temperature inside the cluster rose to 104°F but dropped immediately to 95°F. The bees clustered quietly and there was practically no mortality at the termination of the experiment on January 13. The bees maintained a temperature fluctuating between 91°F to 95°F throughout the period of storage. Temperature outside the cage ranged from 52°F to 70°F. Nine 5-pound cans containing 50 percent sugar syrup were consumed during the nine day period.

2. Two Queen Colony Management. At Madison, Wis., 52 colonies representing 4 stock groups and 4 types of management (package, 2 queen, single queen commercial, and single queen intensive) were studied for production and working time involved. Efficiency in terms of honey produced per unit of time showed the 2-queen system superior to all other systems.

3. Pollen and Longevity. At the Madison laboratory, data from caged nuclei fed supplements made with various aged pollens indicated a decrease in effectiveness of pollens for brood rearing as they age. A formula containing soya flour, brewer's yeast, and dried skim milk supported sustained brood rearing in cages for longer periods than supplements made with pollen.

Pollen collected at weekly intervals in New Jersey and fed to caged bees at the Beltsville laboratory varied greatly in their nutritional value for honey bees as determined by longevity studies.

4. Honey Removal. At Madison, Wis., a large portion of the honey crop was again removed very effectively with high volume low-pressure air blast. Supers were removed in 1/2 to 1 minute with fewer bees remaining in them than by any other method. The air blast generated by a Black and Decker industrial vacuum cleaner has been used the past four seasons. This machine with a 1/4-inch by 2 1/2-inch crevice tool appears to be the best equipment currently available for this purpose.

5. Honey Production vs. Moving of Colonies. A study was made at the Madison laboratory to determine the effect of moving colonies of bees on colony weight loss or gain. The results of these tests and similar tests in 3 previous years indicate that the colonies are not affected by the movement except in the loss of foraging time in re-orienting to new pasture. In practical migratory beekeeping, the loss of foraging time required for re-orientation would be offset by moving into a more favorable foraging area.

6. Hive Cooling and Honey Production. Ten colonies were evaporatively cooled during July and August 1964 in Tucson, Ariz., to determine effects on honey production. During the first three weeks, cooled colonies gained an average of 40.9 pounds each while the uncooled check colonies averaged 25.7 pounds gain. This 59 percent increase in gain was significant at the 5 percent level of probability.

7. Photoperiod Studies. Data obtained from photoperiod studies at Logan, Utah, under controlled environments showed that brood rearing and pollen, nectar and water collecting increased with increasing day length. Decreasing day length led to a rapid decline in brood rearing to a zero point and other signs of preparation for winter developed in the colonies.

8. Apis mellifera adansonii and its hybrids. Observations in Brazil showed that Apis mellifera adansonii hybridizes vigorously and thus has a great impact on local stock. Adansonii and its hybrids sting aggressively and swarm or abscond frequently. The latter tendencies keep the populations low. However, they are exceptionally industrious. A proper breeding program may take advantage of these qualities.

C. Etiology of Bee Diseases and Development of Control Methods for Diseases and Pests

1. Nosema Disease. Nosema disease control is of great economic importance in bee management especially in the production, shipment and use of package bees and queens. Late fall feeding of sugar syrups containing 100 milligrams of fumigillin per gallon fed at the rate of 1 gallon per colony in Madison, Wis., was found to be valuable in delaying the build-up of Nosema

in late winter and early spring. Queen cage candy containing fumigillin was also effective when fed as a mid-winter treatment.

In studies at Baton Rouge, La., the feeding of fumidil B to colonies used for queen cell production reduced but did not eliminate Nosema in these colonies nor did it result in a significant increase in the number of queen cells produced in these colonies.

At Baton Rouge, La., fumigation of combs with glacial acetic acid did not reduce the incidence of Nosema disease in baby nuclei queen mating units.

At Baton Rouge, La., colonies of bees established on new equipment with Nosema inoculated packages of bees showed a higher incidence of disease for the first two months than uninoculated control colonies. After two months, the incidence of the disease reduced to near zero and remained at similar low levels in both groups for the next 10 months. These colonies of bees were headed by artificially mated sister queens to insure genetic likeness between colonies. Queens of the same genotype were provided for similar tests at the Tucson, Laramie, and Madison laboratories.

In this cooperative experiment with the Baton Rouge, Madison, and Tucson laboratories to determine the incidence of Nosema disease in honey bees of the same breeding stock at different geographic locations, inoculated and controlled packaged bees were also established on foundations in new hives at Laramie in May. Sample bees were examined microscopically at 3-week intervals for more than a year. Maximum infections at Laramie occurred about 3 weeks after installation, averaging about 46 percent among inoculated colonies, compared to natural infections of about 11 percent in the control. Nosema decreased rapidly in June.

At Baton Rouge, La., progress has been made in producing Nosema antibodies in inoculated rabbits and preliminary tests indicated that vitamins may effect the development of Nosema disease in colonies of bees.

Repeat studies at Laramie, Wyo., of the effect of humidity upon Nosema disease, showed that sister bees inoculated individually with exactly the same volume of water suspension of Nosema spores developed essentially the same degree of infection whether kept at a high or low percent relative humidity at the same temperature. Apparently humidity does not affect the development of the Nosema parasite once a bee has become infected.

At Laramie, Wyo., phagocytosis of Nosema spores by blood cells was observed microscopically. When these blood cells ingested large numbers of spores they eventually burst perhaps explaining the very low hemocyte counts in Nosema infected bees as opposed to normal bees.

At Beltsville, Md., Nosema spores heated at 120°F for six hours in sugar solution showed greatly reduced or complete loss of viability when tested by individual bee feedings.

2. Disappearing Disease. At Baton Rouge periodic examination of colonies of bees in four areas of Louisiana failed to reveal any pathogen associated with the disappearing disease. In fact, very few colonies in Louisiana showed the symptoms of the disease in 1965 as compared to 1964. Those colonies showing symptoms of the disease were almost totally devoid of stored pollen after the middle of November.

3. American Foulbrood. Field tests for sterilization of American foulbrood contaminated equipment with ethylene oxide were performed at Beltsville, Md. The material used was Benvicide, Matheson, 11% ethylene oxide, 54% freon 11, and 35% freon 12. Seven full depth supers and one shallow containing 8 to 9 frames of dry comb each and well filled with American foulbrood scales were fumigated for 48 hours under a polyethylene tarpaulin. Seven hives were prepared with the fumigated equipment and packages installed. The colonies developed well with excellent brood patterns and no appearance of American foulbrood at any time prior to going into winter.

At Laramie, Wyo., ethylene oxide gas sterilization of combs containing honey and American foulbrood scales was attempted. Cryoxide containing 11% ethylene oxide was used in various concentrations at pressures up to 16 pounds and exposures for as long as 96 hours but full sterilization was not achieved. Ethylene oxide residues in honey were toxic to bees but wax comb did not absorb toxic amounts of the gas.

At the Laramie laboratory honey bee colonies having American foulbrood were treated with the antibiotic tylosin. Gorging bees with sugar syrup containing one gram tylosin lactate per gallon 3 times at about one week intervals eliminated all visible evidence of American foulbrood. The treated colonies have remained healthy for one year. Tylosin lactate is now available commercially as Tylan for control of poultry diseases and indications are that it can be used either in a syrup form or dusting in powdered sugar to aid in control of American foulbrood infections.

At Laramie, Wyo., strains of Bacillus larvae, the bacterium causing American foulbrood, were found resistant to terramycin indicating the danger of B. larvae developing strains resistant to this antibiotic and nullifying its value as an aid in controlling American foulbrood.

4. Autumn Collapse. An outbreak of autumn collapse in late September in California, was investigated by members of the Laramie and Beltsville laboratories at the request of the California authorities. Examination of affected bees revealed no known bee diseases. However, when bee bread obtained from affected colonies was fed by healthy honey bees on a comparison basis with bee bread from unaffected colonies, the bee bread from the affected colonies reduced the survival of the bees by 50%.

5. Bacillus Thuringiensis. At Beltsville, Md., no adverse effects were observed in colonies fed varying concentrations of Bacillus thuringiensis exotoxin and crystals of a nuclear polyhedrosis virus of the corn earworm

and tobacco budworm.

6. Wax Moth. Due to the wide range in recommended dosages of ethylene dibromide (1/2 pound to 11 pounds per thousand cubic feet) for the control of the greater wax moth, tests were made at Beltsville, Md., to determine the minimum dosage for control. One half pound per thousand cubic feet gave 100% control of adult, pupae, larvae, and eggs. The fumigation period was for 24 hours with a temperature range of 20 to 26°C.

7. Conopid Parasites. An unusual conopid fly was found as a parasite of adult honey bees in 5 apiaries near Laramie, Wyo., and Fort Collins, Colo. Three larval stages, the puparium and an adult fly were observed. About 8% of the workers collected between August 30 and September 16 from a dead bee trap in one colony were parasitized. No drones were found parasitized.

Fifty percent of the bees in a sample from Guatemala were found to be harboring conopid parasites upon examination at the Beltsville, Md., laboratory. These parasites were identified as third instar larvae of Zodion sp.

A large number of bumblebee queens and a smaller percentage of bumblebee workers domiciled at the edge of a bee yard at Madison, Wis., were parasitized by a conopid fly.

8. European Foulbrood. In studies conducted by the Beltsville laboratory, the feeding of excess sugar to colonies used for cranberry pollination in southern New Jersey during the period of nectar dearth resulted in a pronounced decrease in the incidence of European foulbrood in these colonies.

9. Sacbrood. At Laramie, Wyo., honey bee larvae of known ages were inoculated by contamination of the food in individual brood cells with filtrates of sacbrood diseased larvae. Larvae from hatching to 4-days old appeared highly susceptible to infection, but larvae of all ages showed some susceptibility to this virus disease.

10. Diseases of Alkali Bee. Twelve species of bacteria were isolated at Laramie, Wyo., from the alkali bee larvae, Nomia melanderi, or from the soil of nesting sites in Riverton, Wyo., Parma, Idaho, or Delta, Utah. Bacteria include, Alcaligenes faecalis, Bacillus cereus, B. circulans, B. laterosporus, B. megaterium, B. pumilis, B. sphaericus, B. subtilis, Brevibacterium insectiphilum, Brev maris, Gaffkya tetragena, and Staphylococcus aureus. All these species were pathogenic to the larvae when inoculated by hypodermic syringe. Their relation to natural infections still remains to be determined.

11. Protozoan Parasites. At Laramie, Wyo., rare protozoan parasites observed microscopically in adult worker honey bees from Louisiana included gregarines found in the midgut of 12 bees and amoebae in the malpighian tubules of a single bee.

12. Hemolymph Studies. At Laramie, Wyo., total hemocyte (blood cell) counts in honey bee larvae of various ages were similar but older pupae showed a higher count. Total hemocyte counts in 5-day-old larvae reared at different geographic locations showed increasing counts with increasing altitudes. Seven distinct types of hemocytes were observed in honey bees. Various coagulation tests with honey bee hemolymph were performed. Although a clot was seldom formed certain similarities with human blood seemed apparent.

13. Virus Disease. At Baton Rouge, La., a disease which kills queens after artificial insemination with sperm from old, confined drones from certain colonies appears a form of virus paralysis. Most diseased queens die a few days after developing swollen abdomens. Some live about 2 weeks. A filtrate prepared from diseased queens has been used to transfer the disease to worker bees.

14. Mites. In work on Acarine disease control under P.L. 480 project E15-ENT-1 in Italy, definite evidence of the acaricide action of menthol on the mite Acarapis woodi was demonstrated in the laboratory. Studies are now in progress to attempt field applications with this chemical. It was also shown that Acarapis woodi mites can survive on the larvae of honey bees for periods up to thirteen days although none of these mites appeared to be well established or able to breed, nor did they enter the larval tracheal system.

Research conducted under P.L. 480 project A7-ENT-10 in India indicates that acarine disease may be limited to certain areas in the northwest Himalayas. If subsequent and more critical survey findings confirm this distribution pattern, a control program for suppression and possible eradication of the mites will be undertaken. Such a program would hold great interest for us if or when American agriculture should face the introduction of this disease. A bonus of this project has been the introduction of the European honey bee, Apis mellifera, into India. Information to date indicates that Apis mellifera is now successfully established in India. This may be a major accomplishment from the standpoint of apiculture in India.

D. Behavior and Utilization of Honey Bees in the Pollination of Agricultural and Other Economic Crops

1. Pheromones. The pheromone 9-oxodec-trans-2-enoic acid was tested at Madison, Wis., for its effect on the behavior of queenless groups of bees. Replicas were established each of queenless, queened, and pheromoned nuclei containing 500 grams of bees. The pheromone was replenished each day. Behavior of the pheromoned colonies was similar to queenright colonies and held their populations well.

2. Bumblebees vs. honey bees. At Madison, Wis., pollen loads were collected from returning field workers of 3 bumblebee species (Bombus fervidus, B. americanorum, and B. auricomus) twice a week for 6 weeks. Concurrently pollen was trapped from 12 honey bee colonies in the same area. The number of pollen gatherers returning to the honey bee colony was also

compared with the number returning to a bumblebee colony. Population counts were made of the honey bee colony and the bumblebee colony each week. Flight range of bumblebees was measured by marking and releasing returning foragers at measured distances from their nests. A range of 1 mile seemed typical for bumblebees as compared with 2 to 3 miles with honey bees. Analyses of pollen samples from the honey bee colonies show 0.58% alfalfa, 14% red clover, and 85% other plant pollens. Analyses of pollen samples from bumblebees showed 24% alfalfa, 56% red clover, and 20% other plant pollens. However, there were at least 5 to 101 times more returning honey bee pollen collectors than bumblebee pollen collectors during any 1-hour period.

3. Repellents and Queen Supersedure. At Madison, Wis., colonies with marked queens were subjected to the repellents acetic acid, propionic anhydride, butyric anhydride, and benzaldehyde 5 days in succession. One month later the colonies were checked for queen supersedure. Four of 10 colonies treated with butyric anhydride had superseded, 2 or 11 treated with benzaldehyde had superseded, but no supersedure occurred in those treated with acetic acid or propionic anhydride.

4. Lipid Components of Pollens. At Tucson, Ariz., 30 species of pollens were analyzed for total saponifiable and nonsaponifiable lipids. All pollens tested were gathered by honey bees except waterleaf (Hydrophilium capitatum) which was collected by Osmia lignaria. Total lipid values were highest for black mustard, and hawksbeard and lowest for salt grass, waterleaf, mule fat, and sweet corn. The saponifiable lipid was highest in black mustard and hawksbeard pollens and lowest in mule fat, waterleaf, salt grass and sweet corn pollen. The nonsaponifiable lipid value ranged from 9.2 milligrams per gram in greasewood and Russian thistle pollens to 53.7 milligrams per gram in hawksbeard pollen. Salt grass had the highest ether extractable material and Russian thistle the lowest.

The saponifiable lipid content and percent distribution of fatty acid in dandelion pollen gathered by bees was also analyzed. When compared with similar analyses of 5 species of hand collected coniferous pollens, dandelion was considerably richer in lipids and in most fatty acids.

5. Landmarks and the Orientation of Bees. An experiment conducted near Logan, Utah, to test the hypothesis that landmarks influenced the degree to which honey bees returned to the same foraging location in a field, indicated that the vertical landmarks supplied were effective in increasing the number of marked bees seen at a location compared to horizontal or no landmarks. The vertical landmarks also seem to increase the population of foraging bees in their immediate neighborhood.

6. Bulk Handling of Bees. At Tucson, Ariz., preliminary tests indicated the possibility of developing techniques for bulk handling of bees. On 2 occasions, 20 and 40 pounds of bees were blown into a large bulk bee screened container, the 40 pound cluster was held for 5 days without

showing signs of distress.

7. Foraging Behavior of Bees on Two Varieties of Cantaloupes. At Yuma, Ariz., bees visited flowers of a "glabrous" line of cantaloupes at the average rate of 7.37 visits per minute compared to 6.44 visits per minute to flowers of the "normal" line. Bees stayed an average of 3.1 seconds on "glabrous" flowers and 6.6 seconds on "normal" flowers. There was noted a tendency for most of the flower to flower movements of the bees to be within the same variety.

8. Pollinating Activity of Honey Bees on Safflower. On a 570-foot-long block of 40 rows of safflower, at Tucson, Ariz., data were collected to see if a diminution of bee activity could be observed as distances from the hives increased. No such forager population gradiant could be seen in this distance although gradiants of bee populations have been seen in larger fields.

The ability of insects other than honey bees to cross pollinate safflower in individual-head cages was tested. Lady bird beetles, lygus bugs, syrphid flies, and sphecid wasps effected virtually no pollination. Halcitus bees, honey bees, and Polistes wasps increased yield and crossing compared to no-insect checks. Polistes wasps particularly show promise as pollinators in small plant cages.

Contamination from outside sources of caged safflower was eliminated by using 20x20-mesh screen cages and by removing all nearby sources of unwanted pollen from around the cages. The latter step alone was effective. The smaller mesh was additional insurance.

9. Bees in Disposable Hives. At Tucson, Ariz., various sizes of clusters of bees were placed in cardboard cartons near alfalfa plots. These "colonies" tended to become more uniform in size based on flight activity and final counts. This was either because of greater mortality in larger populations or because of drifting, although the latter possibility seems remote. Additional comb was built by all but the smallest unit and nectar and pollen were collected and stored.

E. Effect of Pesticides, Insect Diseases, and Farm Practices on Honey Bees and other Pollinating Insects

1. Herbicide Safe for Honey Bees. At Madison, Wis., Glenbar, a new herbicide, was tested on caged bees at various dilutions as a stomach poison and as a contact material. It was concluded that Glenbar is not significally more toxic than 2,4-D and should be a safe material for honey bees when used as recommended.

2. Monitoring. Information gained at Baton Rouge, La., in 1964 on effects of pesticides on honey bees was used to design the 1965 program. This program was changed from one of general observations in 10 areas to the

collection of extensive data in 4 areas. These observations revealed that the pesticides studied did not kill colonies of bees but resulted in occasional heavy losses of field populations following specific pesticide applications. Brood rearing was not affected. Reduced nectar and pollen collection did occur with certain pesticides. Colony recoveries were rapid. Chemical analyses of apiary samples indicated that nectar, honey, royal jelly, queen larvae, worker larvae, drone bees, queen bees, and bee bread were free of pesticides. However, many pollen samples and dead bees from traps showed measurable amounts of pesticides. Future plans include analyses of pollen from specific plant species.

3. Compounds Screened as Honey Bee Attractants and Repellents. At Tucson, Ariz., more than 400 compounds were screened as honey bee attractants or repellents. Numerous compounds produced mixed or variable responses. Some of these responses appeared to be alarm reactions. Sorbaldehyde and sorbic alcohol appeared to be alarm substances not previously reported by other workers. Sorbaldehyde caused the bees to attack and kill one another, particularly when temperatures were high. 5-hepten-2-one-6 methyl was a highly repellent compound for the honey bee, but stimulated the bees to sting observers. Isoamyl acetate and amyl acetate induced bees to approach as to an attractant.

4. Low Volume Malathion Spray Hazardous for Bees. The affects on bees of larger scale aerial applications of low volume malathion (8 fluid ounces per acre) were studied near Sheridan, Wyo. The impact of the malathion treatments could be measured for 7 days on the basis of dead bees recovered on entrance traps. Attempts to protect the colonies by covering them with burlap tarpaulins was partially successful for 2, 4, and 5-day periods. However, unexpected exposures to the insecticide for 2 days prior to covering prevented a full evaluation of covering as a protective technique. The severity of the impact of the malathion appeared to be influenced by nectar flow conditions and the abundance or lack of brood, pollen and honey in the colonies.

5. Pesticides and Bees. At Beltsville, Md., a test for exposing honey bees to experimental insecticides was developed in cooperation with the Pesticides Chemicals Research Branch. Twenty-six compounds were screened by this method in comparison with DDT, carbaryl, and malathion as standards. Four of the compounds were less toxic than DDT. Six of the compounds were less toxic than carbaryl. Of the remaining 16 compounds, 8 were at least as toxic as malathion, and 8 were more toxic than malathion.

6. Pesticides and Alfalfa Pollinators. At Logan, Utah, the LD 50 was determined for each of 15 insecticides for the three most important alfalfa pollinators (Megachile rotundata females, Nomia melanderi females, and Apis mellifera workers). The amounts generally required to produce 50% mortality of M. rotundata females were considerably greater than of the two remaining species.

F. Biology and Utilization of Insects other than Honey Bees in the Pollination of Agricultural Crops.

1. Control of Pests in Leaf Cutter Nests. Triboluim madens were controlled in the nests of Megachile rotundata with the use of DDT treated honey bee pollen pellets in a layer under the nest boxes. Dermestid beetles, (Trogoderma, sp) were controlled in a similar manner.

2. Effect of Temperature and Humidity on Eggs and Larvae of Megachile Rotundata. Below 55% relative humidity the survival rate of eggs and larvae dropped and at 33% and below conditions were lethal. Survival was not as good at fluctuating temperatures as at more constant temperature conditions.

3. Pollinator Collections. In work on wild bee pollinators under P.L. 480 project F4-ENT-4 in Egypt a number and variety of pollinator species were collected in the UAR with particular emphasis on hymenopterus insects in clover fields. Biological studies were also made utilizing three types of artificial nests.

4. Other Species of Apis as Pollinators. Research conducted under P.L. 480 project A7-ENT-19 in India has been concerned primarily with a survey of insect pollinators on different crops and in different agricultural regions of Punjab state. An interesting development is that although Apis florea is the most frequent visitor to Brassica flowers, it is not efficient as a pollinator because it tends to obtain nectar from between the petals without tripping the flower. Apis dorsata although a relatively infrequent bee on Brassica is a more efficient pollinator. Future plans for the project include a similar study of the relation of various native bees to clovers.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Biology and Breeding for Improvement of the Honey Bee

Blum, M. S. and S. Taber III, 1965. Chemistry of the drone honey bee reproductive system - III. Dehydrogenases in washed spermatozoa. J. Insect. Physiol. 11:1489-1501.
Roberts, W. C., 1965. Save-a-step queen rearing. Amer. Bee J. 105:446-47.
Roberts, W. C. and S. Taber, 1965. Egg-weight variance in honey bees. Ann. Entomol. Soc. Amer. 58:303-06.

Management for Improvement in Production of Honey Bees

McDonald, J. L., 1965. Keeping bees in Arizona's desert and irrigated areas. Glean. Bee Cult. 93:464-66.
Moeller, F. E., 1965. Research activities at the North Central States Bee Culture Laboratory. Amer. Bee J. 105:370-71.
Moeller, F. E. and E. R. Harp, 1965. The two-queen system simplified. Glean. Bee Cult. 93(11):679-82, 698.

Etiology of Bee Diseases and Development of Control Methods for Diseases and Pests

Hoage, T. R. and W. C. Rothenbuhler. 1965. Larval honey bee responses to varying doses of American foulbrood spores. J. Econ. Entomol. 59:42-45.
Lehnert, T. 1965. Plural brood in a queenright colony. J. of Apic. Res. 4:99-100.
Shimanuki, H., et. al. 1965. The effect of serial passage of Bacillus larvae white in the honey bee. J. Invert. Pathol. 7:75-78.
Shimanuki, H., P. A. Hartman, and W. C. Rothenbuhler. 1965. In Vitro Growth Studies of Bacillus larvae white. J. Invert. Pathol. 7:437-41.
Whitcomb, Warren, Jr. 1965. Controlling the greater wax moth (Galleria mellonella) a pest of honeycombs. USDA Farmers' Bull. 2217.

Biology and Utilization of Honey Bees in the Pollination of Agricultural and Other Economic Crops

Foster, R. E., M. D. Levin, and S. E. McGregor. 1965. Nectar production by muskmelons infected with four mosiac viruses. Proc. Amer. Soc. Hort. Sci. 86:433-35.
Levin, M. D. 1965. Recent pollination research at the Tucson laboratory. Amer. Bee J. 105:406.
McGregor, S. E. 1966. Summation of the 7th Bee Pollination Conference. Glean. in Bee Culture. 94:18-21, 60.
McGregor, S. E., M. D. Levin, and R. S. Foster. 1965. Honey bee visitors and fruit set of cantalopes. J. Econ. Entomol. 58:968-70.

Sharples, G. C., F. E. Todd, S. E. McGregor, and R. L. Miloe. 1965. The importance of insects in the pollination and fertilization of the Cardinal grape. Proc. Amer. Soc. Hort. Sci. 86:321-25.

Effect of Pesticides, Insect Diseases, and Farm Practices on Honey Bees and Other Pollinating Insects

Cantwell, G. E., D. Knox, T. Lehnert, A. S. Michael. 1966. Mortality of the honey bee, Apis mellifera, in colonies treated with certain biological insecticides. J. Invert. Pathol. 8:228-33.
Johansen, C. A., M. D. Levin, J. D. Eves, W. R. Forsyth, H. B. Busdicker, D. S. Jackson, and L. J. Butler. 1965. Bee poisoning hazard of undiluted malathion applied to alfalfa in bloom. Wash. Agr. Exp. Sta. Cir. 455.

PUBLICATIONS -- STATE EXPERIMENT STATIONS AND COOPERATIVE PROGRAMS

Biology and Breeding for Improvement of the Honey Bee

Benton, A. W., and R. L. Patton. 1965. A qualitative analysis of proteins in the venom of honey bees. J. Insect. Physiol. 11:1359-64. (N.Y.)
Laidlaw, H. H., and K. W. Tucker. 1965. Three mutant eye shapes in honey bees. J. Hered. 56:190-92. (Calif.)
Tucker, K. W., and H. H. Laidlaw. 1965. Compound inseminations to abbreviate tests for allelism in honey bee queens. J. Hered. 56:127-30. (Calif.)

Etiology of Bee Diseases and Development of Control Methods for Diseases and Pests

Girardeau, J. H., Jr. 1965. The occurrence of dry-mix-fed drugs in larval food of the honey bees. J. Econ. Entomol. 58:878-80. (Ga.)

Effect of Pesticides, Insect Diseases, and Farm Practices on Honey Bees and other Pollinating Insects

Graves, J. B., and Otto Mackensen. 1965. Topical application and insecticide resistance studies on the honey bee. J. Econ. Entomol. 58:990-3. (La.)
Shaw, F. R., R. A. Callahan and F. R. Holbrook. 1965. The effectiveness of MGK 6091 as a repellent to bees visiting alfalfa bloom. Amer. Bee J. 105:137. (Miss.)

Biology and Utilization of Insects other than Honey Bees in the Pollination of Agricultural Crops

Brander, N. R., R. V. Frakes and W. P. Stephen. 1965. Effects of bee species and isolation distance on possible varietal contamination in alfalfa. Agron. J. 57:247-48. (Oreg.)

Cazier, Mont A. and Martin A. Mortenson. 1965. Studies on the bionomics of sphecoid wasps. IV. Nitelopterus laticeps and Nitelopterus texanus (Hymenoptera: Sphecidae). Pan-Pacific Entomol. 41:21-9. (Calif.)

Cazier, Mont A. and Martin A. Mortenson. 1965. Studies on the bionomics of sphecoid wasps. V. Bothynostethus distinctus and Entomognathus texana (Hymenoptera: Sphecidae). Pan-Pacific Entomol. 41:30-3. (Calif.)

Cazier, Mont A. and Martin A. Mortenson. 1965. Studies on the bionomics of sphecoid wasps. VI. Pernaldina lucae (Hymenoptera: Apoidea). Pan-Pacific Entomol. 41:34-43. (Calif.)

Mathewson, J. A. 1965. The internal morphology of the eastern cucurbit bee Peponapis pruinosa (Hymenoptera: Apoidea). J. Kansas Entomol. Soc. 38:209-22. (R.I.)

Stephen, W. P. 1965. Temperature effects on the development and multiple generations in the alkali bee, Nomia melanderi Ckll. Entomol. Exp. Appl. 8:220-28. (Oreg.)

Stephen, W. P. 1965. Effects of soil moisture on survival of prepupae of the alkali bee. Nomia melanderi Ckll. J. Econ. Entomol. 58:472-74. (Oreg.)

Stephen, W. P. 1965. Artificial beds for alkali bee propagation. Oregon Agr. Exp. Sta. Bull. 598. 29. (Oreg.)

Stephen, W. P. and C. E. Osgood. 1965. Influence of tunnel size and nesting media on sex ratios in the leaf-cutter bee, Megachile rotundata (Fabr.) J. Econ. Entomol. 58:965-68. (Oreg.)

Stephen, W. P. and C. E. Osgood. 1965. The induction of emergence in the leaf-cutter bee, Megachile rotundata (Fabr.), and important pollinator of alfalfa. J. Econ. Entomol. 58:284-86. (Oreg.)

AREA NO. 16. ANALYSIS, SYNTHESIS, FORMULATION, AND EVALUATION OF INSECT CONTROL CHEMICALS

Problem. Modern insecticides are a rapid and effective means of controlling injurious insects and their use has enabled the American farmer to produce an abundance of high quality crops and livestock. This extensive use, however, has been accompanied by increasing resistance of some insects to certain insecticides and by the possibility of leaving harmful residues on or in harvested crops, in meat, or in dairy and poultry products. There is therefore a need for the development of new types of chemicals, from natural sources and through synthesis, to which insects will not become resistant. These chemicals should be safe to handle and not leave harmful residues in products used for foods or feeds, or adversely affect wildlife, beneficial insects, and other desirable organisms. More effective and safer formulations of chemicals should be developed for the control of different insect species under various environmental conditions. Such chemicals and formulations require initial testing in the laboratory and evaluation under field conditions before they can be recommended for practical use. It is essential that accurate, sensitive analytical methods be developed for the determination of the amounts of chemicals deposited and the rate of disappearance of their residues and breakdown products in treated crops, animals, or soils. Better attractants as well as lures for additional important insect pests are needed for use in traps and bait sprays for both insect detection and control. Research also is needed on repellents that would be useful in controlling insect attacks on crops, livestock, and man. Insect chemosterilants appear promising for use in insect control and their potentialities should be thoroughly explored.

USDA AND COOPERATIVE PROGRAM

The Department has a long-term program of basic and applied research involving chemists, biochemists, entomologists, and scientists of other specialized disciplines to discover, evaluate, and develop new and improved types of insect control chemicals and methods of utilizing them. Chemical research to discover, isolate, and identify products of natural origin which can be employed for insect control is carried on mainly at Beltsville, Md. Components of the cotton plant that act as attractants, arrestants, feeding stimulants, essential nutrients, or otherwise affect the boll weevil are being investigated at State College, Miss., in cooperation with the Mississippi Agricultural Experiment Station. An investigation of the natural sex attractant of the codling moth is in progress at Yakima, Wash. Grants have been made to the University of Michigan for research on the sex attractant of the tobacco budworm and to the University of Wisconsin for research on the tobacco hornworm. Chemical research on synthetic organic materials and formulations for insect control is carried on at Beltsville, Md.; Gainesville, Fla., and State College, Miss. Contracts have been negotiated with the Midwest Research Institute in Kansas City, Mo., for the synthesis of intermediate compounds needed in the research on insect attractants, repellents and chemosterilants, and with the Hazleton Laboratories, Inc., in Falls Church, Va., for

determination of the mammalian toxicity of candidate chemosterilants, attractants, or other new types of insect control agents. Development of analytical methods for insecticide residues is carried on at Beltsville, Md.; Tifton, Ga.; Kerrville, Tex.; Yakima, Wash., and College Station, Tex. There is cooperation with the State Experiment Stations in the respective regions of these laboratories. Cooperative work with the States on insecticide residues is conducted in connection with the following Regional Research Projects: NC-85, Reduction of Hazards Associated with the Presence of Residues of Insecticidal Chemicals in the Environment; NE-36, Pesticide Residues in or on Raw Agricultural Commodities; NE-53, Transformation of Insecticides by Plants; S-22, Agricultural Chemical Residues in Plant and Animal Products; W-45, Pesticide Residues; Their Nature, Distribution, and Persistence in Plants, Animals, and Soils; IR-4, Evaluation of Current Data and Needed Research to Obtain Clearance for Safe, Effective Chemicals for Minor Uses on Agricultural Products. Research on aerosols for insect control is conducted at Beltsville, Md. Biological evaluation of insecticides and other types of insect control chemicals is carried on at Beltsville, Md., and Brownsville, Tex. Research on methods for control of insects in aircraft is done at Beltsville, Md.

The Federal scientific effort devoted to research in this area totals 45.6 scientist man-years. Of this number 11.8 are devoted to products of natural origin as sources of insect control materials; 17.8 to development of synthetic organic materials and formulations for insect control; 5.8 to methods of analysis for insecticide residues; 1.0 to aerosols for insect control; 7.0 to biological evaluation of insect control materials; 0.2 to methods for control of insects in aircraft; and 2.0 to program leadership.

In addition, the Federal support of research under contracts and grants provides 3.3 man-years in this area. Of this total 1.5 is devoted to products of natural origin as sources of insect control materials and 1.8 to development of synthetic organic materials and formulations for insect control.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 33.0 professional man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Products of Natural Origin as Sources of Insect Control Materials

1. Insect Sex Attractants. Chemical research on the isolation and identification of the natural sex attractants of important insect species is being carried on at Beltsville, Md. The molecular structure of the pink bollworm sex attractant, which was identified last year, has now been verified by synthesis. The attractant, 10-propyl-trans-5,9-tridecadienyl acetate, was made by a 13-step synthesis and has been given the name propylure. It is thought to be the first naturally occurring compound with propyl branching to be

reported. The synthetic compound is of the same order of attractancy for adult male pink bollworm moths as is the attractant isolated from the virgin female moths.

Chemists at Beltsville have developed an improved method of synthesis for the sex attractant of the cabbage looper, cis-7-dodecenyl acetate, which was first identified and synthesized by investigators at Auburn University. The synthetic material has shown excellent attractancy for male cabbage looper moths in laboratory and field tests.

A large batch of gyplure (the synthetic gypsy moth sex attractant developed by Beltsville chemists) that had been freed of ricinoleyl alcohol was very effective in gypsy moth trap drop tests conducted by the Plant Pest Control Division. It failed, however, to compete with live females under natural conditions in a confusion test.

Good progress has been made at the Yakima, Wash., laboratory on the extraction, isolation, and purification of the codling moth sex attractant.

The phenomenon of attractant masking has been demonstrated at Beltsville for both the cynthia moth and the American cockroach sex attractants. Crude extracts of the bodies of females of these species failed to elicit any sexual response from the males of the respective species. In each case, a highly attractive fraction could be separated from the extract by column chromatography on a suitable adsorbent. In the case of the cynthia moth, admixture of this active fraction with one of the inactive fractions resulted in complete inactivity; admixture with each of the other fractions failed to mask the activity. This was in contrast to the behavior of the American cockroach sex attractant, in which the attractancy of the active fraction could be masked only by admixture with the combined inactive fractions.

An olfactometer has been developed for use in demonstrating the attractiveness of females of the yellow mealworm to the males of this species. It will be used in studying the natural sex attractant of this species.

Under the grant to the University of Michigan for research on the sex attractant of the tobacco budworm, satisfactory methods were developed for isolation of the pure sex attractant from abdominal tips of the virgin female moths and for bioassaying active fractions behaviorally on live male moths in the laboratory. Progress of the work was hampered by the small numbers of insects available, which delayed isolation of sufficient pure attractant for identification.

Under the grant to the University of Wisconsin for research on the sex attractant of the tobacco hornworm, methods were developed for breaking diapause, collecting the attractant from live virgin female moths, and testing it behaviorally in the laboratory. Progress was made in the isolation of the pure attractant.

2. Materials of Plant Origin. At Beltsville, Md., echinacein, the insecticidal constituent of American coneflower root, has been identified as N-isobutyl-trans-2,cis-6,trans-8, trans-10-dodecatetraenamide. Echinacein, a highly unstable compound, was isolated in impure form from roots of the American coneflower (Echinacea angustifolia) at Beltsville in 1954, but was not identified because of lack of material. It has now been re-isolated from that plant as well as the roots of E. pallida. It is identical with alpha-sanshool and neoherculin, isolated previously from the bark of Zanthoxylum species, members of a completely unrelated plant family. Echinacein is as effective as pyrethrins against house flies and causes the same rapid knockdown. However, it polymerizes in the air after one hour at room temperature, and in a nitrogen atmosphere after two days at 10°C.

At State College, Miss., in studies on components of the cotton plant that attract or stimulate feeding by the boll weevil, investigation of the carbonyl compounds in cotton bud oil was completed. Forty-four carbonyl compounds were found present and 14 of these were positively identified, accounting for 88% of the carbonyl fraction, which in turn constituted 1.4% of the square (cotton bud) oil. The compounds identified were: acetone, isobutyraldehyde, butyraldehyde, isovaleraldehyde, hexanal, heptanal, trans-2-hexenal, nonanal, 2-octenal, benzaldehyde, 2-nonenal, trans-2-cis-6-nonadienal, p-tolualdehyde, and myrtenal. Of particular interest were trans-2-cis-6-nonadienal, the principal odor ingredient in cucumbers; trans-2-hexenal, leaf aldehyde, the principal defense secretion among many insects; and myrtenal, derived from alpha-pinene which is a principal terpene constituent of the cotton bud oil.

A study of the phospholipids of the cotton bud also was completed. The phospholipids were characterized as to nitrogen base, phosphorus content, ester titer, and fatty acid composition. The fatty acid distribution in the cotton bud lecithins and the degree of unsaturation of the fatty acids in the neutral lipids were studied. It was found that 70% of the total phospholipid phosphorus appeared in the amino-nitrogen-free and choline-containing phospholipids. Linoleic acid is the major acid in the alpha-position of the cotton bud lecithins, while equal amounts of linoleic and linolenic acid are in the beta-position. The alpha-position has 24 mole percent and the beta-position 4 mole percent of palmitic acid as the only saturated fatty acid.

Separation of cotton seed oil into fractions of various chemical classes and study of the boll weevil feeding stimulation and attractancy of these fractions is in progress. Preliminary results show that the protein and phospholipid fraction has strong feeding activity but no insect attractancy. A second source of activity was found in the neutral fraction. Column chromatography of the neutral fraction on silicic acid with pentane, chloroform, chloroform-methanol, and methanol yielded a chloroform fraction that had both feeding activity and attractancy.

B. Development of Synthetic Organic Materials and Formulations for Insect Control

1. Preparation of Synthetic Organic Compounds for Testing as Insect Chemosterilants. C^{14}-Labeled hempa (hexamethyl phosphoramide) was synthesized at Beltsville by the reaction of dimethylamine with phosphorus oxychloride. This radioactively labeled material was used in a study of the degradation and metabolism of hempa in house flies (See E. 2).

A study was made of the ring-opening reaction of aziridinylbis(dimethylamino)-phosphine oxides to elucidate the relationship of structure to reactivity. The ring-opening process is considered to be a necessary step in the physiologically important reaction of the aziridinyl insect chemosterilants. The study included additions to 1-aziridinyl-, 2-methyl-1-aziridinyl-, and 2,2-dimethyl-1-aziridinylbis(dimethylamino)phosphine oxides.

A new type of insect chemosterilant containing boron was discovered. Several organoboron compounds were found to have chemosterilant activity, especially toward screw-worm flies.

Under contract with the Midwest Research Institute of Kansas City, Mo., 9 compounds were received at Beltsville in moderate quantities for use as test materials or intermediates for further synthesis in the chemosterilants program.

Chemical studies on the effects of C^{14}-tepa in the boll weevil were continued at State College, Miss.

At Gainesville, Fla., the studies of the mechanism of apholate resistance in Aedes aegypti mosquitoes and the relationship of sterility to hydrocarbons present in cockroach haemolymph were still in progress.

2. Preparation of Synthetic Organic Compounds for Testing as Insecticides, Insect Attractants or Repellents, or Synergists. A simple method has been devised for determining the volatility of insect attractants. This makes it possible to predict the length of time required for complete evaporation of an attractant and also permits comparison of the volatility of different attractants. The comparative volatility of trimedlure (an attractant for the Mediterranean fruit fly) and its isomers was determined in this way. It was shown that the heats of fusion of the isomers can be related to the volatility. The secondary butyl isomer would take about twice as long as trimedlure itself (the tertiary butyl isomer) to evaporate. Other insect attractants also were compared by this means.

Samples of butyl sorbate, an attractant for the European chafer, that had been stored for one year were found to show some deterioration. It was found that this material could be salvaged by distillation from hydroquinone. Various

antioxidants are being investigated as additives to prevent deterioration of the European chafer attractant, butyl sorbate, on storage.

Caproic acid and related acids have been found to be highly attractive to both sexes of Olcella parva (Adams). This species is not of economic importance, however.

In connection with research at Beltsville on the molecular structure of compounds that are of interest for insect control, a new inexpensive ozonizer was developed that is very useful for determining the positions of double bonds in minute samples of organic compounds. Technique for ozonizing as little as one microgram of material was developed with applicability to various types of structures. An apparatus also was devised for instantaneously saturating multiple bonds with hydrogen in connection with carbon skeleton chromatography.

In fulfillment of their contract Midwest Research supplied 34 materials in quantities of from 2 to 1000 g for use as attractants or repellents or as intermediates for the synthesis of these materials.

3. Formulations. Improvements were made at Beltsville in apparatus and procedures for measuring the flowability of pesticide powders, to provide better mechanical standardization and reduce potential interference from static charges. The value of the angle of repose as an index of powder flowability was investigated and the correlation was found to be poor. In cooperation with the Cooperative Pesticide Analytical Committee of Europe, the information obtained in this work was applied to the modification of a proposed CPAC method.

In research at Beltsville, Md., on improved formulations, ultra-low-volume sprays and conventional emulsion sprays were compared in aerial and ground applications on cotton foliage. Although ground application of emulsifiable concentrates gave smaller residue than aerial application at the same rate, there was little difference in the magnitude or persistence of malathion deposited from the air as undiluted technical material or as the 57% emulsifiable concentrate and no difference in residue from undiluted and diluted emulsifiable concentrates of methyl parathion.

At College Station, Tex., studies are in progress on systemic insecticides formulated for slow release of toxicant into the stem of the cotton plant. A simple laboratory procedure has been devised which gives a rough index of the rate of diffusion of Azodrin-C^{14} from oil to aqueous phases. Three oils (or mixtures) found to give large differences in diffusion rates will be used in bio- and radioassay tests in the greenhouse in order to correlate laboratory and greenhouse procedures. The effects of viscosity, concentration, distribution coefficients, absorbents, and thickening agents on diffusion rates are being investigated.

At Gainesville, Fla., formulations of deet, benzyl benzoate, and M-1960 were used to impregnate new cotton army fatigue uniforms to determine the absorption of these repellents in wet and dry treatments. Greater recovery of repellent was obtained when the garments were first rinsed in cold water and then wrung to remove the excess prior to application of the formulations.

4. Testing of Respiratory Protective Devices. Testing is being continued on the effectiveness of various respirator cartridges or filters in the removal of pesticide particles from an injected airstream. The publication "Respiratory Devices for Protection Against Certain Pesticides" (ARS 33-76-2) was revised and issued and has received wide distribution and acceptance.

C. Methods of Analysis for Insecticide Residues

At Beltsville a gas chromatographic method has been developed for the determination of residues of dimethoate and its oxygen analog at the 0.01 ppm level in forage and milk.

Infrared spectrophotometry is the basis of an analytical method developed for residues of demeton and its insecticidal metabolites in recovery studies on apples, spinach, string beans, tomatoes, and potatoes.

A colorimetric procedure to determine hempa based on its phosphorus content gives good recovery immediately following injection into house flies. An extract of untreated flies produced absorbance no greater than the reagent blank.

Cooperation between workers at Beltsville, Md., and Tifton, Ga., resulted in the construction of a new type of extraction apparatus for the rapid equilibration of solvent phases in the cleanup of pesticide residue extracts prior to analysis.

Other work at Tifton, Ga., included a gas chromatographic method developed for the determination of residues of Azodrin (R) (3-hydroxy-N-methyl-cis-crotonamide dimethyl phosphate) and Bidrin(R) (the N,N-dimethyl analog) in raw extracts of sweet corn. The flame photometric detector used can detect as little as 2 parts per billion without undue interference from the corn extract. The method is being used to follow persistence of Azodrin in the field.

A similar technique was devised for determining residues of Imidan(R) (O,O-dimethyl S-phthalimidomethyl phosphorodithioate) and its phosphorothioate analog, Imidoxan, in corn silage and sweet corn. After prior separation of the 2 compounds on a silica gel column, chromatographic analyses using the flame photometric detector recorded as little as 2 and 4 parts per billion for Imidan and Imidoxan, respectively.

Also developed at Tifton were gas chromatographic procedures for the analysis of the chemosterilants tepa, metepa, methiotepa, hempa, and apholate to about 0.1 nanogram. Adult fall armyworms fed tepa-C^{14}-tepa in sucrose solution were subjected to analysis at intervals thereafter by radiometric and gas chromatographic methods. A comparison of the tepa content of external rinses, homogenates, and excreta of the insects demonstrated that radiometric analysis is not satisfactory for the determination of tepa per se.

Studies were made on the fluorescence and phosphorescence of 12 carbamate pesticides and 1-naphthol. Intensity of fluorescence decreased in order as follows: 1-naphthol, carbaryl, Matacil(R) (4-(dimethylamino)-m-tolyl methylcarbamate), Bay 39007 (o-isopropoxyphenyl methylcarbamate), Mobil MC-A-600 (benzo[b]thien-4-yl methylcarbamate), and Zectran(R) (4-(dimethylamino)-3,5-xylyl methylcarbamate). Phosphorescent intensities decreased in the following order: Mobil MC-A-600, Pyrolan(R) (3-methyl-1-phenylpyrazol-5-yl dimethylcarbamate), and Chloro IPC (isopropyl m-chlorocarbanilate). No appreciable response was obtained under the stated conditions with 8 other generally tested carbamates.

An analytical method using spectrofluorescence was developed for the determination of Velsicol AR-50 and the synergists piperonyl butoxide or sulfoxide in mixtures containing pyrethrins, Deobase, and DDT in the proportions usually found in aerosol sprays. The method was extended to piperonyl butoxide residues in multiwall paper bags, rice, flour, and cornmeal in cooperative work between the Tifton laboratory and the Insects Research and Development Laboratory of the Market Quality Research Division at Savannah, Ga.

At College Station, Tex., a simple procedure has been devised to assay Azodrin(R) (3-hydroxy-N-methyl-cis-crotonamide dimethyl phosphate) based on diffusion rates. (See B. 3).

At Beltsville, Md., a number of samples were analyzed for pesticide content to assure conformity with current standards.

At Kerrville, Tex., gas chromatography has been used to develop a method for determining residues of Dursban(R) (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) in animal tissues.

The fluorescence of coumaphos and carbaryl, which can be intensified by treatment with alkali, has been used to determine residues of these materials on animal hair. A steer was sprayed with 0.25% coumaphos and photographic records were made in a light-proof room using high intensity ultraviolet light and fast film. The slow disappearance over 4 days of the initial strong fluorescence has considerable promise in the future evaluation of spray methods and formulations.

At Yakima, Wash., a procedure to determine residues of Niagara NIA-10242 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) based on iodination is being developed.

D. Aerosols for Insect Control

Some 10% allethrin aerosols in containers 1 inch in diameter and 4 inches high were sent to Viet Nam for test to determine whether these small containers were practical. The reports sent back were enthusiastic with the additional comment that the spray was effective in removing leeches from soldiers.

A one-shot 5% dichlorvos aerosol which vaporizes on release was developed for eradication of cockroaches in enclosed spaces. Good results are obtained under the proper conditions (See E. 3). Requirements for the valve, can, and formulation have been drafted.

A fiber-drum spray has been devised for small scale field or laboratory tests which simulates particle sizes produced by conventional airplane and ground spraying. For field use the drums are inverted over the plants to be treated and the liquid pesticide is atomized through a 1-inch hole in the inverted bottom. Exact dosages can be applied by means of a syringe which measures and injects the pesticide into the nozzle. Depending upon the gas pressure used in the atomizer, particle sizes from the aerosol range up to 100 microns m.m.d. can be produced.

E. Biological Evaluation of Chemicals for Insect Control

1. Insecticides. A major activity in this area is the laboratory testing of synthetic organic compounds and natural products against representative species of insects to determine whether the materials have insecticidal, synergistic, attractant, repellent, insect chemosterilant, growth controlling, or other effects that would be useful for insect control. Preliminary evaluation tests on these materials are carried out at Beltsville, Md., and Brownsville, Tex., by the Pesticide Chemicals Research Branch and at 21 other locations by other Branches of the Division, throughout the United States and in Mexico, on 65 insect and 8 mite species. Some of the materials tested originate within the Pesticide Chemicals Research Branch and many others are supplied by other government or private research agencies and by industry. These materials are also submitted for evaluation to the Stored Products Insects Research Branch, Market Quality Research Division, laboratory at Savannah, Ga., in cooperative research with this Division.

A total of 488 compounds from 45 different industrial or government sources were evaluated in the laboratory for insecticidal or acaricidal activity, 235 compounds from 7 industrial sources for attractancy, and 657 compounds from 52 industrial or government sources for repellency.

A new annual report was developed for internal use in the Department on the results of field evaluations of candidate insecticides and acaricides conducted by Division laboratories. This report is in the form of a qualitative synopsis. A total of 121 materials received from 28 industrial sources were evaluated in the field. A number of these compounds showed considerable promise as broad spectrum insecticides. Preliminary data indicate that some of these compounds may have very favorable mammalian toxicities.

2. Materials That Control the Activities of Insects Through Effects Other Than Death. A study of the metabolism of C^{14}-tepa and its mechanism of sterilization in male house flies, Musca domestica, has been concluded with the following results: A wet combustion method of determining C^{14}-labeled compounds was developed which give reproducible and quantitative recovery of the radioactive material. The radioactivity of a dose of C^{14}-tepa adjusted to approximately 1 microgram per male fly and administered by injection was almost totally recovered from the treated flies and their excreta. Respiratory $C^{14}O_2$ during the first 12 hours amounted to only 0.75%. After 5 hours of normal activity, 50% of the injected dose was still retained by the fly in the form of tepa or aziridinyl metabolites and approximately 9% and 5% persisted for prolonged periods as determined by radiometric and colorimetric methods, respectively. The radioactivity detected in the excreta was due to non-aziridinyl metabolites. Approximately 1% of the radioactivity was transmitted to the female fly, presumably by copulation with the treated male since the activity resided within the female body rather than on the surface. However, no radioactivity was detected in the sperm present in the spermathecae of inseminated females. An electron microscopic scrutiny of the house fly sperm failed to reveal any structural changes resulting from the tepa treatment.

In experiments using C^{14}-hempa with a specific radioactivity of about 100 mc/mM it was found that the metabolism of hempa proceeds 1.4 times faster than for tepa at the 50% recovery level. An extract of house flies injected with hempa when subjected to gas chromatography revealed one metabolite which will be studied further. The in vivo reaction rate of hempa is 9 times slower than tepa when compared at the 50% sterility level.

In a related study a dose of 3.12 microcuries of hempa per male fly, equivalent to 5.7 micrograms, the ED_{50} for hempa, was used to inject the insects. Recovery of radioactivity was determined on both flies and excreta. When flies were confined following treatment, 50% of the injected dose could be recovered from the fly after 5 hours. At the end of 24 hours, only 15% of the dose was retained in the fly and 75% was in the excreta for a total recovery of 90%. Only 1.25% could be recovered as $C^{14}O_2$ after 24 hours.

When the dose injected was increased to 29.4 micrograms of hempa with a slightly smaller activity of 2.82 microcuries per fly and the flies were allowed normal activity the disappearance curve showing recovery from the flies declined more abruptly than in the previous experiment. Fifty percent of the

injected dose could be recovered 2-1/2 hours after treatment and at the end of 24 hours only 3% was still retained in the fly.

The American cockroach, its severed head, and the remainder of the body were assayed for the presence of the sex pheromone by using single males selected for high sensitivity as an indicator. The pheromone is a sexual stimulus released by nymphs and adults of both sexes; however, it is produced in much greater amounts by maturing females. It occurs mainly in the head and is probably secreted by modified areas of the cuticle.

Experiments were made with an artificial antenna made from cotton thread soaked with Ringer solution to determine whether polarizable electrodes contribute to the shape and kinetics of the electroantennagram (EAG) of insects. Platinum electrodes were used to compare the EAGs from artificial antennae with those from cockroach antennae. The effect of a capillary pulled over one or both electrodes was also studied. Although artificial EAGs are obtained under certain conditions, they differ in response curve, shape, and kinetics from EAGs of insect antennae.

3. Aerosols and Space Sprays. A new Japanese chrysanthemumate, Neopynamin(R) (2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylic acid ester with N-(hydroxymethyl)-1-cyclohexane-1,2-dicarboximide), is generally superior to pyrethrins and allethrin in knockdown of house flies and of comparable effectiveness in kill at an aerosol concentration of 2%. Performance improves with increasing concentration up to 5%. Aerosols containing 3% or more of Neopynamin alone were as effective as the G-1357 Federal Specification Aerosol (parathion 0.6%, piperonyl butoxide 1.4%). In comparing formulations of the material with piperonyl butoxide, it was more effective than allethrin, less effective than pyrethrins in kill of resistant house flies. It was superior to both allethrin and pyrethrins in aerosol combinations with DDT. In spray tests it was effective against both resistant and susceptible house flies and several species of mosquitoes. Against cockroaches Neopynamin aerosols were not as good as allethrin or pyrethrins, but the sprays were comparable to pyrethrins and caused greater knockdowns.

Against face flies, the sprays were much more effective than pyrethrins. The effectiveness of residue tests may be summarized as follows: against face flies, greater than DDT, slightly less than malathion; against resistant house flies, less than malathion; against German cockroaches, nearly equal to malathion after 4 weeks. It was also effective against the box elder bug and 2 of 3 species of crickets. Neopynamin appears promising as an insecticide due to its significant knockdown. Oral toxicity for rats is reported to be 5200 mg/kg.

An aerosol containing 1% allethrin and 1% dichlorvos was effective against resistant house flies at a dosage of 5 grams per 1000 cubic feet. A formulation containing 1.2% allethrin and 6% dimethrin was as effective against resistant house flies as the Federal Specification Aerosol that contains 0.6% pyrethrins, 1.4% piperonyl butoxide.

A one-shot 5% dichlorvos aerosol which vaporizes on release gave 100% kill of German cockroaches when delivered at the rate of 100 grams of formulation per 1000 cubic feet. The vapor must be contained in an enclosed space so that the roaches are exposed for one hour or more to obtain good results.

In laboratory tests of residual treatments for cockroach control, 10 mg/ft^2 of Mobil MC-A-600 (benzo[b]thien-4-yl methylcarbamate) killed 100% of males of the brown-banded cockroach in 2 hours after the residue had aged for 80 weeks. It was still 100% effective in 4 hours after 124 weeks. A similar residue of Monsanto CP-40115 (O-2-chloroethyl ethylphosphonothioate O-ester with p-hydroxybenzonitrile) killed 100% of adult male German roaches in 5 hours after 24 weeks and was still giving 80% kill in 3 days when the residue had aged 1 year.

Four strains of resistant German cockroaches were selected out of 6 strains available for a proposed mixture of chlordane-resistant strains to be distributed to members of the Chemical Specialties Manufacturers Association. These strains ranged from 0 to 50% kill after 72 hours exposure to chlordane residues of 10 milligrams per 1000 square centimeters.

The relative tolerance of 16 species of cockroaches to residues of malathion was studied. The most susceptible species tested were Supella supellectillium, Nauphoeta cinera, and Eurycotis floridana. The most resistant species were Periplaneta brunnea and P. americana. In tests of susceptibility to chlordane residues Blaberus discoidalis appeared more susceptible than B. cranifer and B. gigantius which had been previously tested.

Tests with the Official Test Aerosol against smoky-brown cockroach, Periplaneta fuliginosa, showed that this species was about as susceptible to the OTA as P. americana, which is more susceptible than P. australasiae and P. brunnea. P. brunnea is the most tolerant.

Face fly densities on livestock were lower in Maryland than in previous years. A single treatment of a beef herd with 1% dimetilan sugar-bait spray caused fly population reductions. Daily treatment of dairy herds with sugar-bait sprays containing 1% Ciodrin$^{(R)}$ (alpha-methylbenzyl 3-hydroxycrotonate dimethyl phosphate) also caused reductions in moderate face fly populations.

A dust containing 2% dimetilan tested against horn flies by baiting beef cattle to feeding stations almost completely eliminated the pest for 8 or 9 weeks. A 5% coumaphos treatment was less effective.

F. Methods for Control of Insects in Aircraft

The study of aerosol formulations for aircraft disinsection made at Beltsville in cooperation with the WHO has been published in the Bulletin of the World Health Organization. It was found that both the G-1492 aerosol used in the U. S. and the formula proposed by the Pyrethrum Board of Kenya (pyrethrum, sulfoxide, and isopropyl myristate) were highly effective against the resistant Trinidad strain of Aedes aegypti as well as house flies.

G. Radar Tracking of Insects

The radar tracking of insects was explored in cooperative research with the Johns Hopkins University Applied Physics Laboratory and the Air Force Cambridge Research Laboratory as part of a study of radar "echoes" from an apparently clear atmosphere. In initial tests at Wallops Island, Virginia, adults of the tobacco hornworm, corn earworm, and honey bee were released individually at 8,000 feet altitude from an aircraft locked in the range gate of the radar unit. When the insect appeared on the screen as a second blip the radar was locked on the insect for tracking its free flight. Positive separation of all 3 insects from the aircraft was recorded and tracking patterns were obtained for the tobacco hornworm and corn earworm moths giving information on the flight path of the insect, speed of flight, changing altitude of flight, wing-beat frequencies and periods of rest. Locking difficulties cast some doubt on tracking data obtained for the honey bee. It appears that radar could be a valuable tool in obtaining hitherto unavailable entomological data.

Since calculations involved in the tracking study required information on the water content of insects, a number of different species were assayed by macerating the insect in anhydrous methanol and titrating with Karl Fischer reagent. Insects analyzed included Apis mellifera, Melanoplus femurrubrum, Protoparce sexta, Musca domestica, Diabrotica undecimpunctata howardi, Lygus lineolaris, Euchistus serus, Hypera punctata, Hoppodamia convergens, Hypera postica, Epilachna varivestis, Periplaneta americana, Heliothis zea, Periplaneta brunnea, Eurycotis floridana, Periplaneta australasiae, Leucophaea maderae, Nauphaeta cinerea, Blaberus craniifera, Heliothis virescens. Water content ranges from 50 to 76%.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Products of Natural Origin as Sources of Insect Control Materials

Fletcher, L. W., J. J. O'Grady, Jr., H. V. Claborn, and O. H. Graham. 1966. A pheromone from male screw-worm flies. J. Econ. Entomol. 59: 142-3.

Graham, H. M., J. M. McGough, and M. Jacobson. 1966. Influence of solvent on effectiveness of sex lure for pink bollworm. J. Econ. Entomol. 59: 761-2.

Hedin, P. A., A. C. Thompson, and J. P. Minyard. 1966. Constituents of the cotton bud. III. Factors that stimulate feeding by the boll weevil. J. Econ. Entomol. 59: 181-5.

Jacobson, M. 1965. Isolation and characterization of insect attractant lipids. J. Amer. Oil Chem. Soc. 42: 681-5.

Jacobson, M., and L. A. Smalls. 1966. Masking of the American cockroach sex attractant. J. Econ. Entomol. 59: 414-6.

Jones, W. A., M. Jacobson, and D. F. Martin. 1966. Sex attractant of pink bollworm moth: isolation, identification, and synthesis. Science 152: 1516-7.

Knapp, J. L., P. A. Hedin, and W. A. Douglas. 1965. Amino acids and reducing sugars in silks of corn resistant or susceptible to corn earworm. Ann. Entomol. Soc. Amer. 58: 401-2.

Minyard, J. P., J. H. Tumlinson, P. A. Hedin, and A. C. Thompson. 1965. Constituents of the cotton bud. Terpene hydrocarbons. J. Agr. and Food Chem. 13: 599-602.

Thompson, A. C., and P. A. Hedin. 1966. Separation of organic acids by thin-layer chromatography of their 2,4-dinitrophenylhydrazide derivatives and their analytical determination. J. Chromatog. 21: 13-18.

Development of Synthetic Organic Compounds for Testing as Insect Control Agents

Acree, F., Jr., R. B. Turner, B. J. Smittle, and G. S. Burden. 1965. Hydrocarbons in haemolymph of cockroaches of different ages. J. Insect Physiol. 11: 905-10.

Beroza, M., F. Acree, Jr., R. B. Turner, and B. H. Braun. 1966. Separation and insect repellent activity of diastereoisomers of 2-ethyl-1,3-hexanediol against Aedes aegypti. J. Econ. Entomol. 59: 376-8.

Beroza, M., and R. Sarmiento. 1965. New catalyst and technique for analyzing fatty acids, their esters, and long-chain compounds by carbon-skeleton chromatography. Anal. Chem. 37: 1040-1.

Berry, I. L., M. L. Beadles, and H. V. Claborn. 1966. The distributions of coumaphos on cattle from limited-volume mist sprays and one-gallon saturating sprays. J. Econ. Entomol. 59: 347-52.

Borkovec, A. B., and C. W. Woods. 1965. Insect chemosterilants. II. N-carbamoylaziridines. J. Med. Chem. 8: 545-7.

Chang, S. C. 1965. Chemosterilization and mating behavior of male house flies. J. Econ. Entomol. 58: 699-72.

Chang, S. C. 1965. Improved bioassay method for evaluating the potency of chemosterilants against house flies. J. Econ. Entomol. 58: 796.

Chang, S. C., and A. B. Borkovec. 1966. Determination of tepa residues on chemosterilized Mexican fruit flies. J. Econ. Entomol. 59: 102-4.

Fletcher, L. W., J. J. O'Grady, Jr., H. V. Claborn, and O. H. Graham. 1966. A pheromone from male screw-worm flies. J. Econ. Entomol. 59: 142-3.

Harrell, E. A., M. C. Bowman, and W. W. Hare. 1965. An effect of electrostatic dusting on DDT dust deposition. J. Econ. Entomol. 58: 1016-7.

McGovern, T. P., and M. Beroza. 1966. Structure of the four isomers of the insect attractant trimedlure. J. Org. Chem. 31: 1472-7.
Schwartz, P. H., Jr., and R. B. Turner. 1966. A dietary study of the adult eye gnat, Hippelates pusio (Diptera: Chloropidae). Ann. Entomol. Soc. Amer. 59: 277-80.
Settepani, J. A., and A. B. Borkovec. 1966. Heterocyclic amines. A convenient synthesis of 3,5-diamino-1,2,4-triazine derivatives. J. Heterocyclic Chem. 3: 188-90.
Valega, T. M. 1966. Nuclear magnetic resonance study of some N,N-dimethylcarbamates. J. Org. Chem. 31: 1150-3.
Yeomans, A. H. 1965. Inhalation hazard. Agr. Chem. 20: 56-7, 61.
Yeomans, A. H., R. A. Fulton, Floyd F. Smith, and Ruth L. Busbey. 1966. Respiratory devices for protection against certain pesticides. USDA, ARS 33-76-2, 10 p.

Methods of Analysis of Insecticide Residues

Beck, E. W., L. H. Dawsey, D. W. Woodham, and D. B. Leuck. 1966. Dimethoate residues on soybean, corn, and grass forage. J. Econ. Entomol. 59: 78-82.
Beroza, M., and M. C. Bowman. 1966. Apparatus and procedures for rapid extraction and identification of pesticides by single and multiple distribution in binary solvent systems. Anal. Chem. 38: 837-41.
Bowman, M. C., and M. Beroza. 1965. Analysis of Imidan colorimetrically and by electron-affinity gas chromatography. J. Assoc. Off. Agr. Chem. 48: 922-6.
Bowman, M. C., and M. Beroza. 1965. Extraction p-values of pesticides and related compounds in six binary solvent systems. J. Assoc. Off. Agr. Chem. 48: 943-52.
Bowman, M. C., M. S. Schechter, and R. L. Carter. 1965. Behavior of chlorinated insecticides in a broad spectrum of soil types. J. Agr. Food Chem. 13: 360-5.
Bowman, M. C., H. C. Young, and W. F. Barthel. 1965. Minimal concentrations of aldrin, dieldrin, and heptachlor in soil for control of white-fringed beetles as determined by parallel gas chromatographic and biological assays. J. Econ. Entomol. 58: 896-902.
Claborn, H. V., H. D. Mann, I. L. Berry, and R. A. Hoffman. 1965. Comparisons of residues in milk resulting from two types of spray applications of DDT, Shell Compound 4072, and ronnel. J. Econ. Entomol. 58: 922-3.
Claborn, H. V., and M. C. Ivey. 1965. Determination of O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate in animal tissues and milk. J. Agr. Food Chem. 13: 353-4.
Claborn, H. V., and M. C. Ivey. 1965. Determination of 2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate and 2,2',4'-trichloroacetophenone in animal tissues and milk. J. Agr. Food Chem. 13: 354-6.
Fahey, J. E., J. W. Butcher, and R. T. Murphy. 1965. Chlorinated hydrocarbon insecticide residues in soils of urban areas, Battle Creek, Michigan. J. Econ. Entomol. 58: 1026-7.

Fahey, J. E., R. T. Murphy, and R. D. Jackson. 1965. Residues of chlorinated hydrocarbon insecticides found on corn plants treated for European corn borer control. Iowa State J. Sci. 39: 437-47.

Ivey, M. C., H. V. Claborn, R. A. Hoffman, O. H. Graham, J. S. Palmer, and R. D. Radeleff. 1966. Residues of Shell Compound 4072 in the body tissues of sprayed cattle. J. Econ. Entomol. 59: 379-82.

Jackson, R. D., Jack E. Fahey, and R. T. Murphy. 1965. Residues of carbamate insecticides on corn plants treated for European corn borer control. Iowa State J. Sci. 39: 449-52.

Walker, K. C., Donald A. George, and Jay C. Maitlen. 1965. Residues of DDT in fatty tissues of big game animals in the states of Idaho and Washington in 1962. USDA, ARS 33-105, 21 p.

Walker, K. C., J. C. Maitlen, J. A. Onsager, D. M. Powell, and L. I. Butler. 1965. The fate of aldrin, dieldrin, and endrin residues during the processing of raw sugarbeets. USDA, ARS 33-107, 8 p.

Aerosols for Insect Control

Adler, V. E., A. H. Yeomans, and E. S. Fields. 1965. Low-volume aerosol ground insecticide applicator. J. Econ. Entomol. 58: 780-1.

Yeomans, A. H. 1965. Low-volume spraying technique shows promise for future. Croplife: 13-14.

Yeomans, A. H. 1966. Low-volume ground spray. Agr. Chem. 21: 27-9.

Biological Evaluation of Chemicals for Insect Control

Fales, J. H., and O. F. Bodenstein. 1965. New household insecticide. Soap and Chem. Spec. 41: 91-4, 123-4.

Onsager, J. A., B. J. Landis, and H. W. Rusk. 1966. Control of wireworms on potatoes in eastern Washington by soil fumigants and organophosphorus insecticides. J. Econ. Entomol. 59: 441-3.

Stuerckow, B. 1965. The electroantennogram (EAG) as an assay for the reception of odours by the gypsy moth. Ins. Physiol. 2: 1573-84.

Woodrow, A. W., Nathan Green, H. Tucker, M. H. Schonhorst, and K. C. Hamilton. 1965. Evaluation of chemicals as honey bee attractants and repellents. J. Econ. Entomol. 58: 1094-1102.

Young, J. R., and M. C. Bowman. 1966. Evaluation of Shell SD-8447 for control of two sweet corn insects. J. Econ. Entomol. 59: 170-3.

Methods for Control of Insects in Aircraft

McGuire, J. U., Jr., W. N. Sullivan, and Roger Starkweather. 1966. Analysis of the effectiveness of aircraft-disinsection aerosols against resistant house flies and mosquitoes. Bull. World Health Organ. 34: 151-4.

PUBLICATIONS -- STATE EXPERIMENT STATIONS AND COOPERATIVE PROGRAMS

Bache, C. A., and D. J. Lisk. 1965. Determination of organophosphorus insecticide residues using the emission spectrometric detector. Anal. Chem. 37: 1477-80. (N. Y.)

Crosby, D. G., E. Leitis, and W. L. Winterlia. 1965. The photodecomposition of carbamate insecticides. J. Agr. Food Chem. 13: 204. (Calif.)

Eberle, D. O., and F. A. Gunther. 1965. Chromatographic, spectrometric, and irradiation behavior of five carbamate insecticides. J. Assoc. Off. Agr. Chem. 48: 927-37. (Calif.)

El-Refai, Ali, and T. L. Hopkins. 1965. Thin layer chromatography and cholinesterase detection of several phosphorothiono insecticides and their oxygen analogs. J. Agr. Food Chem. 13: 477-81. (Kans.)

Gunther, F. A., T. A. Miller, and T. E. Jenkins. 1965. Continuous chloride-ion combustion method applied to determination of organochlorine insecticide residues. Anal. Chem. 37: 1386-91. (Calif.)

Gutermann, W. H., and C. A. Bache. 1965. Determination of Imidan and Imidoxon in crops by preparative thin layer and by gas chromatography. J. Gas Chromatog. 2: 350-2. (N. Y.)

Harrendorf, Keith. 1965. A modified high-clearance sprayer for plot use. J. Econ. Entomol. 58: 1014-15. (Mo.)

Ott, D. E., and F. A. Gunther. 1965. DDD as a decomposition product of DDT. Residue Reviews 10: 70-84. (Calif.)

Szkolnik, M., K. D. Hickey, E. J. Broderick, and D. J. Lisk. 1965. Mercury residue of apple fruit as related to application schedules and to colorimetric and neutron activation analysis. Plant Diseases Rptr. 568-79. (N. Y.)

AREA NO. 17. IDENTIFICATION OF INSECTS AND RELATED ARTHROPODS

Problem. Only about a third of the estimated two million or more kinds of insects in the world have been described and named. Many of these are of no known immediate concern to agriculture or mankind, but thousands of species are known to be or are potentially destructive or useful. Minute morphological differences are very important in recognizing many species, and only highly trained specialists are able to postively identify known species and describe new ones. Precise information on the identity and distribution of insects is essential to the efficient conduct of programs concerned with research on harmful insects and the development of methods for their control and in the management of regulatory activities intended to exclude, control, or eradicate insect pests.

Knowledge of the classification and identification of insects at present is far from adequate. Knowledge of the insect fauna of the world provides the best assurance that any potential pests will be immediately recognized so that appropriate safeguards can be set up to exclude them or prompt action taken to control or eradicate them if accidentally introduced. Moreover, with increasing emphasis on the utilization of beneficial insect parasites and predators to help control destructive insects, it is necessary that we know which insects to search for, where they might be found, and how to recognize those that may be useful.

USDA AND COOPERATIVE PROGRAM

The program of the Department is a long-continuing one involving insect taxonomists. It includes basic research to make known to science previously unrecognized and undescribed species of insects, ticks, and mites and the application of the results of this research to the problems of insect identification. The work is carried on to a limited extent at Beltsville, Maryland, but mostly at two locations in Washington, D. C., in close cooperation with the U. S. National Museum of the Smithsonian Institution. Cooperation, close but somewhat less active, is maintained with various centers of taxonomic research in the United States and in foreign countries and with numerous individuals in many parts of the world.

The Federal scientific effort devoted to research in this area totals 25.0 scientist man-years. Of this number, 7.5 are devoted to basic studies to name and describe beneficial and injurious insects, mites, and ticks; 10.9 to the identification of insects, mites, and ticks; 6.1 to the preparation of keys and monographs on the classification, distribution, morphology, and biology of insects and related arthropods; and 1.5 to program leadership.

In addition, Federal support of research in this area by means of 3 grants and 1 contract provides 1.1 man-years devoted to basic studies to name and describe beneficial and injurious insects. One of the grants is to Cornell University for basic studies on the taxonomy, morphology, and ecology of

cutworm larvae. Another is to North Carolina State University at Raleigh for basic studies on the nature and taxonomic significance of morphological characters of female leafhoppers. The third grant, only very recently initiated at Rutgers University, involves basic studies on the morphology of insect sense receptors stimulated by attractants. Research on the contract comprises a taxonomic study of the North American weevils related to the boll weevil, and of various populations of the boll weevil itself, by the Agricultural Experiment Station of Texas A&M University.

Research in this area is also conducted under nine P. L. 480 projects. In Uruguay, S9-ENT-6 provides for 1.5 man-years devoted to the classification of grasshoppers; and in Colombia, S5-ENT-2 has 2 man-years devoted to a biochemical study of Drosophila classification. Five projects are operating in India as follows: A7-ENT-24 provides 3 professional man-years for a systematic study of thrips; A7-ENT-28 provides 2.25 man-years for taxonomic studies of Mallophaga (biting lice); A7-ENT-29 provides 2 man-years for a taxonomic study of Bruchidae (seed beetles); and A7-ENT-37 provides approximately 2.5 man-years for a taxonomic survey of parasitic Ichneumonidae in India. A project in Egypt, F4-ENT-2, provides 4 professional man-years for a study of the insect fauna of Egypt. In Pakistan, A17-ENT-10 provides 1.5 man-years on leafhopper taxonomy.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 21.2 professional man-years is devoted to this area of research.

PROGRESS - USDA AND COOPERATIVE PROGRAM

A. Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites, and Ticks.

1. Cockroaches. Two new cockroaches, one from tropical America and one from West Africa, were described as a service to British entomologists. These cockroaches are commonly intercepted in shipments from these two general areas to the British Isles.

2. Grasshoppers. Research has been continued on the active program to discover and make known the grasshoppers of Uruguay and nearby areas. This research is supported by a P. L. 480 grant. Many thousands of specimens have been collected, and during the year, comparisons have been made between many of them and reference material at United States and European museums.

3. Two-winged Flies. The world-wide genus, Atherigona, contains many species that are saprophytes. Several species, however, cause primary damage to living plants. Wheat plants in West Pakistan were recently found to be attacked by one of the latter type, and the species was described to provide a name for use by economic entomologists involved with insect problems in the Orient.

The Chaoboridae are close relatives of mosquitoes and aquatic midges and are relatively well studied in North America. Two new species, however, were discovered during the year and were described and named. In other fly families, new species in such relatively well understood but unfamiliar genera as Parabezzia, Eumecosomyia, Stenomicra, and Pygophora were discovered in connection with more extensive revisionary work.

The black flies are universally known to be pests of man. A new species of the genus Simulium, from Southwestern United States, was described.

Biochemical data gathered through P. L. 480-supported research on some 20 species of vinegar flies (Drosophila) in Colombia, South America, have been published or reported. Optical intensities of five to ten fluorescent compounds in most of the species studied show measurable differences between the species, differences that may serve to aid in the classification of vinegar flies.

4. Thrips. The Hood collection of thrips, one of the most important single, private collections of insects in the United States, was moved to Washington from New York State. The great many types, many of them representing exotic species, make the collection especially valuable for studies of species occurring in foreign countries and for research by visiting scientists.

Publications of research results from a P. L. 480 project in India include descriptions of species new to science and records of previously unknown geographical distribution of thrips. Work on the project has also resulted in the development of a collection of more than 15,000 thrips mounted on microscope slides. While most of these remain in India, valuable material of a good percentage of mounted specimens has been deposited in the U.S. National Collection of Insects.

5. Beetles. A new genus and species of Anobiidae from Jamaica was described in both the larval and adult stages in continuing taxonomic studies of species in that family that occur in the Caribbean, and several new species were described in the anobiid genus Tricorynus from the United States and Mexico.

An undescribed species of the seed-weevil (Bruchidae) genus Algarobius was received from the Canadian National Museum for identification and description. Nearly all of the 1,350 specimens were reared from mesquite. Another seed weevil, Algarobius prosopis, is a common Mexican species which makes up nearly one-third of the bruchids intercepted along the Mexican border.

As a result of a P. L. 480 project located in India, eight heretofore undescribed species of seed beetles were collected, bringing the total of species known from that country to 37. Eleven of these attack edible seeds, some of which are widely used, while the others breed in pods of wild plants. Taxonomic studies are being conducted on larvae; the larval stage of 29 species are known so far.

Research on the taxonomy of the boll weevil, being continued on contract with the Texas Agricultural Experiment Station, has enabled specialists to evaluate more fully some previously studied morphological characters which distinguish the boll weevil from its near relative on wild cotton, the thurberia weevil. A preliminary key to distinguish the pupae of 42 species of Anthonomus and related genera has also been devised, and work continues to elucidate larval characters as well. The larvae of about 30 species in the tribe Anthonomini are now known.

6. Aphids. Another very important private collection, numbering 1,000 specimens of aphids, was combined with the Smithsonian collections. Represented are about 125 species, some by type material, more than half of which were previously not identified or poorly represented in the collections in Washington.

7. Leafhoppers. Thirty-three previously undescribed species of leafhoppers belonging to a number of genera, four of which are also new to science, have been discovered in Pakistan as a result of studies being conducted there under a P. L. 480 grant. Work on other species shows that some Pakistan typhlocybine leafhoppers are of probable economic importance in feeding on three important trees in that country.

Preliminary results of research on ovipositors of leafhoppers being conducted through a grant to North Carolina State University show that the second valvulae of ovipositors continue to demonstrate promising structural characters. Research is being conducted to determine if other characters furnish supporting or refuting evidence for the preliminary conclusions.

8. Moths. Research on larvae of the cutworm moths (Noctuidae), being conducted on a grant to Cornell University, has provided larvae of 23 species that have been associated with adults through rearing. Plants, mostly trees and shrubs, that serve as hosts for the larvae, have been determined. A sizable number of color transparencies of living larvae has been accumulated.

9. General. Our knowledge of the insect fauna of the Galapagos Islands is fragmentary. However, the University of California Extension's two-year expedition to those islands, sponsored by the Charles Darwin Foundation, has resulted in the accumulation of a large collection of insects, many of which have never been described. The collection has now been sorted to family and is being referred to specialists around the world. Several taxonomists of this Branch have produced revisions of insect groups, on which they are specialists, using this material for study. The entire identification effort is expected to yield a great deal of information about speciation and distribution of insects.

The survey of insects of Egypt has completed its fourth year as a P. L. 480 project. Since its beginning, approximately 25,000 specimens representing all the insect orders and families known to occur in Egypt were collected and are being mounted, labeled, and studied. A number of specimens have been identified by USDA and outside specialists, and additional material

is being submitted for identification and study as its preparation is completed.

USDA specialists are participating in a comprehensive biological survey of the island of Dominica, British West Indies, which is being conducted by a continuing Smithsonian-Archbold Expedition. Two Branch taxonomists have surveyed the island, each for a period of three months, collecting insects of especial interest to their research. The continuing survey will enable those who participate to make intensive field studies of the biologies of the insects with which they work taxonomically, thus enabling them to make more intelligent taxonomic decisions.

B. Identification of Insects, Mites, and Ticks.

Authoritative identifications and references to pertinent taxonomic and biological literature are supplied to support Federal and State research, extension, control, and regulatory activities pertaining to entomological problems. These services are also performed for industry, pest control operators, and private individuals in the United States and for foreign agencies and institutions concerned with entomology.

During the year, a total of 34,244 lots of insect material was received for identification. About 360,158 specimens were examined. A total of 82,234 identifications was made and reported. Specimens were accepted for identification only when rendering the service could be justified, as there is a backlog of material awaiting study.

The sources of material and the numbers of identifications made of the specimens received from each are shown in the following table:

Source	Number of Identifications	Percent of Total
Agricultural Research Service		
Plant Quarantine Division	25,232	30.75
Plant Pest Control Division	2,495	2.98
Entomology Research Division	3,444	4.17
Forest Service	1,322	1.61
Agricultural Marketing Service	150	0.17
Other Federal Agencies	2,798	3.40
States and Insular Possessions	29,860	36.26
U. S. individuals	8,090	9.82
Foreign agencies and individuals	8,879	10.84
Total determinations	82,270	100.00

Many of the specimens received for identification are of much interest, either representing new species not previously in the National Collection or documenting new distributional and/or other data. For these reasons,

84,405 specimens of especial value were added to the National Collection during the year.

The systematic review of technical literature essential to the programs in this area included the examination of 1,924 publications which contained 5,660 articles of interest to insect taxonomists. Reference (by author) cards to these articles totaled 8,899. A total of 2,212 articles was cataloged in depth, and from this effort 21,692 file cards were made up, on which data of significance to taxonomists were recorded. The cards are in continual use in research and service activities, and the file for each specialist is kept up-to-date and immediately available to him.

During the year, 89 visitors obtained aid on taxonomic, nomenclatural, and other problems. The visitors remained for varying periods of time, from an hour or so to several weeks, and came from all parts of the world.

C. Preparation of Keys and Monographs on the Classification, Distribution, Morphology, and Biology of Insects and Related Arthropods.

1. Two-winged Flies. A "Catalog of Diptera of America North of Mexico" has been published as Agriculture Handbook No. 276. The volume of 1,696 pages lists all the names ever proposed for North American Diptera. There are 1,971 valid generic and 16,130 valid species names as opposed to the previous, most recent catalog published in 1905, which treated 952 genera and 5,432 species. The volume, in preparation for about five years, was organized and edited by the specialists of the Taxonomic Investigations of Diptera Unit. All of the Branch specialists on flies were among the 50 or so contributors of specific fly families that comprised the body of information given in the catalog. It will be invaluable to everyone engaged in research and in regulatory phases of entomology and has already stimulated wide interest abroad.

All of the scientists in the Taxonomic Investigations of Diptera Unit have been invited to participate in the production of "A Catalog of the Diptera of the Americas South of the United States," a venture sponsored by the Department of Zoology in the Office of the Secretary of Agriculture, Sao Paulo, Brazil. Each specialist will submit catalogs of families in his assignment. Several such manuscripts have been already published, and others are in preparation. One of the Branch dipterists has written the preface to the series.

During the previous year, a key to the mosquitoes of Vietnam was published by the U.S. Army Surgeon General's Office of Research and Development as the result of a long-term research project on contract with the Smithsonian Institution. A revision of this key was published during the year to update this very necessary information in the light of developments in that country.

It is not often that revision of an entire fly family appears in print between two covers. However, the fly family Tanypezidae was so treated

during the year. Members of this family are rarely encountered, and the literature, now reviewed in a single paper, heretofore had been extremely difficult to obtain for review.

The study of type-specimens and type-species is vital to the understanding of the taxonomy and proper recognition of insect groups. The types of Tephritidae (fruit flies) and Sciomyzidae (snail flies) described by early workers, such as Walker, van der Wulp, and Loew, were examined principally in the British Museum (Natural History) by Branch scientists. Likewise, a type-species was proposed for the genus Chamaemyia of the family Chamaemyidae, and lectotypes of a number of Panamanian Drosophila were discussed and described.

Keys, distributional notes, and nomenclatural and taxonomic observations have been made in a number of fly groups. The third larval instar and puparium of Odontoloxozus longicornis, a neriid intercepted at quarantine, was described for more ready recognition by inspectors. Remarks on various phases of the systematics of mosquitoes were presented in publication. Various genera in the fly families Otitidae, Anthomyzidae, Chloropidae, Sylvicolidae, and Ceratopogonidae were revised with keys, descriptions, and illustrations.

2. Wasps. A key to the species of the eulophid genus Elasmus now enables workers to distinguish the various forms in this important and ubiquitous group of parasites, some species of which are primary, some secondary, parasites. Species of Pediobius, another eulophid genus, are primarily secondary parasites; but a few are primary in Diptera boring in the stems of grasses, and two are important primary parasites of the Hessian fly. A revision of the North American species of Pediobius appeared almost simultaneously with a revision of Palaearctic species published in Czechoslovakia, now enabling specialists to identify all the known species that occur in the northern hemisphere.

3. Sawflies. To assist field survey crews in identifying the European apple sawfly and the pear sawfly, a pictorial key to larvae and adults of both of these introduced insects, accompanied by descriptive text, was published. The pear sawfly was thought to have been detected in North America many years ago, but has not been seen again until quite recently, and is very similar in appearance to the European apple sawfly which is commonly encountered in its range in North America.

4. Moths. For the first time recently, the beet armyworm, Spodoptera exigua, was recorded from Ohio and Florida. Even more recently, larvae of this insect were collected from cotton leaves in La Calera, Managua, Nicaragua. The species, not previously known to occur south of Mexico, is apparently extending its range southward. Studies such as these add materially to our knowledge of insect movements, distribution, and eventually, aid in our discoveries of how evolutionary processes might have taken place.

Additional observations, mostly nomenclatural, have been made in this group of insects. As our knowledge of insects grows, studies must be continually made on the correct application of names. Such studies have been made on the corn earworm and on several genera among other families, especially in the microlepidoptera.

5. Mealybugs. A series of revisionary papers on this group of insects is continued with a fifth contribution, which details changes occurring in past years in our concepts of seven mealybug species, some of economic importance.

6. Beetles. The khapra beetle was at one time one of America's most important introduced pests. To review its characters and to distinguish them from those of a closely related species, Phradonoma tricolor, which is commonly intercepted at American ports of entry, a description of the larvae of the latter, along with biological notes, was published.

An illustrated key to species of the genus Dermestes, another prominent group of beetles affecting stored products, was made available.

Taxonomic and other revisionary work on species of the beetle family Anobiidae has been carried on as part of a long-term research project. The anobiid genus Tricorynus was completely revised with keys and illustrations to aid species identification. Taxonomic notes were also presented to clarify the status of the seed-weevil genus Abutiloneus. The identity and biology of an aspen root girdler, Agrilus horni, was elucidated in the course of continuing studies on insects affecting this group of trees.

7. Mites. Because of continuing interest in mites in connection with various biological control projects and because the family Tydaeidae had never before been treated taxonomically as a unit, a review of the genera was produced. The tydaeids are common on plants where other mites abound. Some are plant feeders, and others are predators; but their existence in these circumstances makes it mandatory to have a means of identifying them. A few species are known to be economically important pests in other parts of the world.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAM

Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites, and Ticks.

Ananthakrishnan, T. N. 1965. Thysanopterologica Indica - III. Entomol. Tidskrift 86:49-63, illus.

Ananthakrishnan, T. N. 1965. Gall-inhabiting tubuliferous Thysanoptera - I. J. Bombay Nat. Hist. Soc. 62:266-78, illus.

Ananthakrishnan, T. N. 1966. Thysanopterologica Indica - IV. Bull. Entomol. 7:1-12, illus.

Gurney, A. B. 1965. Two new cockroaches of the genera Pelmatosilpha and Henschoutedenia, with a key to the West Indian species of Pelmatosilpha (Dictyoptera: Blattaria). Proc. Royal Entomol. Soc. London (B) 34:5-11, illus.

Hoenigsberg, H. F., L. E. Magalhaes, A. J. Chejne, and E. Bautista. 1965. Biochemical studies of Drosophila systematics II; III. Universidad de los Andes Estudios Genetica.

Marsh, P. M. 1965. Two species of Microgasterinae new to the United States. Proc. Entomol. Soc. Wash. 67:214.

Steyskal, G. C. 1965. Records and descriptions of African Otitidae and Platystomatidae (Diptera). Ann. Nat. Museum 18:171-78, illus.

Steyskal, G. C. 1966. A new species of Atherigona Rondani causing damage to wheat plants in West Pakistan. Proc. Entomol. Soc. Wash. 68:53-56, illus.

Steyskal, G. C. 1966. Notes on the genus Pygophora Schiner, with the description of one new species (Diptera: Muscidae). Pacific Insects 8: 14-16, illus.

Stone, Alan. 1965. Two new Chaoboridae from the United States (Diptera). Proc. Entomol. Soc. Wash. 67:231-33, illus.

Stone, Alan, and M. M. Boreham. 1965. A new species of Simulium from the Southwestern United States (Diptera, Simuliidae). J. Med. Entomol. 2: 164-70, illus.

Todd, E. L. 1966. A new species of Opsigalea Hampson from Texas. Proc. Entomol. Soc. Wash. 68:149-51, illus.

White, R. E. 1966. A new genus and new species of Anobiidae from Jamaica with a larval description (Coleoptera). Coleopterist's Bull. 20:60-63, illus.

Wirth, W. W. 1966. A new Jamaican blood-sucking midge from lacewings (Diptera, Ceratopogonidae). Proc. Entomol. Soc. Wash. 68:29-32, illus.

Identification of Insects, Mites, and Ticks.

Burks, B. D. 1965. The North American Species of Elasmus Westwood (Hymenoptera, Eulophidae). Proc. Biol. Soc. Wash. 78:201-207, illus.

Burks, B. D. 1966. The North American species of Pediobius Walker (Hymenoptera, Eulophidae). Proc. Entomol. Soc. Wash. 68:33-43, illus.

Foote, R. H. 1965. A study of the types of Tephritidae described by F. M. van der Wulp in "Biologia Centrali-Americana" (Diptera). J. Kansas Entomol. Soc. 38:236-47.

Foote, R. H. 1966. Notes on the types of Tephritidae described by R. W. Doane (Diptera). Proc. Entomol. Soc. Wash. 68:120-26.

Ford, E. J., Jr., and J. M. Kingsolver. 1966. Description and biological notes on the larvae of Phradonoma tricolor (Coleoptera: Dermestidae). Coleopterist's Bull. 20:27-29, illus.

Herring, J. L. 1965. The status of Amphiareus Distant, Buchananiella Reuter and Poronotellus Kirkaldy (Hemiptera: Anthocoridae). Proc. Entomol. Soc. Wash. 67:202-203.

Herring, J. L. 1966. The correct name for an anthocorid predator of the Cuban laurel thrips (Hemiptera: Anthocoridae). Proc. Entomol. Soc. Wash. 68:93.

Hodges, R. W. 1965. Generic names in Recurvaria group. Entomol. News 76: 262-64.

Hodges, R. W. 1965. Comments on names of some North American moths. Coop. Econ. Ins. Rep. 15:1294.

Hodges, R. W. 1966. The use of the name Udea. Coop. Econ. Ins. Rep. 16:5.

Kingsolver, J. M. 1965. A key to the species of the genus Dermestes (Dermestidae) commonly intercepted in USDA plant quarantine inspections. Plant Quarantine Mimeograph Ident. Aid 4 pp., illus.

Kingsolver, J. M. 1965. On the genus Abutiloneus Bridwell (Coleoptera: Bruchidae). Coleopterist's Bull. 19:125-28, illus.

McKenzie, H. L., and D. J. Williams. 1965. Fifth taxonomic study of North American mealybugs, with revisional notes on seven species (Homoptera: Coccoidea: Pseudococcidae). Hilgardia 37:1-15, illus.

Nord, J. C., F. B. Knight, and G. B. Vogt. 1965. Identity and biology of an aspen root girdler, Agrilus horni. Forest Sci. 11:33-41, illus.

O'Neill, Kellie. 1966. A new synonym of Taeniothrips eucharii (Whetzel) (Thysanoptera: Thripidae). Proc. Entomol. Soc. Wash. 68:5.

Pipkin, S. B., and W. W. Wirth. 1965. Lectotypes of Panama Drosophila (Diptera, Drosophilidae). Proc. Entomol. Soc. Wash. 67:147-48.

Russell, L. M. 1966. A note on the names Adelges and Adelginae (Homoptera: Aphidoidea). Coop. Econ. Ins. Rep. 16:193.

Sabrosky, C. W. 1965. Diptera-Chloropidae, in Mission zoologique de l'I.R.S.A.C. en Afrique orientale. Ann. Museum Roy. Afr. Centr., Zool. 138:401-12.

Sabrosky, C. W. 1965. Asiatic species of the genus Stenomicra (Diptera: Anthomyzidae). Bull. Brit. Museum (Nat. Hist.), Entomol. 17:209-18, illus.

Sabrosky, C. W. 1965. Chamaemyia Meigen. 1803 (Insecta, Diptera, Chamaemyiidae); proposed designation of a type-species under the plenary powers. Bull. Zool. Nomenclature 22:267-68.

Smiley, R. L. 1966. Further descriptions of two Erythraeids predaceous upon cotton bollworm eggs (Acarina:Erythraeidae). Proc. Entomol. Soc. Wash. 68:25-28, illus.

Smith, D. R. 1966. The Nearctic sawflies of the genus Hemitaxonus Ashmead (Hymenoptera: Tenthredinidae). Proc. Entomol. Soc. Wash. 68: 113-20, illus.

Smith, D. R. 1966. Recognition of the European apple sawfly and the pear sawfly (Hymenoptera: Tenthredinidae). Coop. Econ. Ins. Rep. 16:228-30, illus.

Spilman, T. J. 1965. Ptinus variegatus in Georgia and Kentucky (Coleoptera: Ptinidae). Proc. Entomol. Soc. Wash. 67:230.

Spilman, T. J. 1966. On the generic names Alphitobius, Phtora, Clamoris, and Cataphronetis (Coleoptera: Tenebrionidae). Proc. Entomol. Soc. Wash. 68:6-10.

Steyskal, G. C. 1966. Notes on types of some species described in Sciomyza and Tetanocera by Loew, Walker, and van der Wulp (Diptera: Sciomyzidae, Muscidae, Neriidae, Pyrgotidae). Studia Entomol. 8:445-48, illus.

Steyskal, G. C. 1965. The third larval instar and puparium of Odontoloxozus longicornis (Coquillett) (Diptera, Neriidae). Ann. Entomol. Soc. Amer. 58: 936-37, illus.

Steyskal, G. C. 1966. The genus Eumecosomyia Hendel (Diptera, Otitidae). Proc. Entomol. Soc. Wash. 68:100-102.

Steyskal, G. C. 1966. A case of homonymy in the genus Hercostomus (Dolichopodidae). Proc. Entomol. Soc. Wash. 68:169.

Stone, Alan. 1965. Notes on Nearctic Sylvicola species. Proc. Entomol. Soc. Wash. 67:149-51.

Stone, Alan, and J. Hamon. 1965. Remarques sur la systématique des moustiques (Culicidae s. str.). Cahiers O.R.S.T.O.M. Ent. Méd. Nos. 3 and 4:3-9.

Todd, E. L. 1965. A note on the generic nomenclature of the corn earworm and its relatives (Lepidoptera: Noctuidae). Coop. Econ. Ins. Rep. 15:1328.

Todd, E. L. 1966. A new distribution record for the beet armyworm, Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae). Coop. Econ. Ins. Rep. 16:5.

White, R. E. 1965. Taxonomic and distribution notes on Anobiidae (Coleoptera). Coleopterist's Bull. 19:113-16.

White, R. E. 1965. A revision of the genus Tricorynus of North America (Coleoptera: Anobiidae). Misc. Pub. Entomol. Soc. Amer. 4:285-368, illus.

Wirth, W. W. 1965. A revision of the genus Parabezzia Malloch (Diptera, Ceratopogonidae). Proc. Entomol. Soc. Wash. 67:215-30, illus.

Preparation of Keys and Monographs on the Classification, Distribution, Morphology, and Biology of Insects and Related Arthropods.

A Catalog of the Diptera of America North of Mexico. 1965. USDA, Agr. Handbook 276.
Coulson, J. R., C. W. Sabrosky, and Irmgard Muller. Selected bibliography of North American Diptera. pp. 1117-1484.
Foote, R. H. Family Cecidomyiidae. pp. 241-95.
Foote, R. H. Family Tephritidae. pp. 658-78.
Foote, R. H. Family Rhinotoridae. pp. 818-19.
Foote, R. H. Family Ropalomeridae. p. 679.
Foote, R. H. Family Cryptochetidae. p. 825.
Foote, R. H., J. R. Coulson, and Harold Robinson. Family Dolichopodidae. pp. 482-530.
Sabrosky, C. W. Family Diopsidae. pp. 637-38.
Sabrosky, C. W. Family Periscelididae. p. 710.
Sabrosky, C. W. Family Milichiidae. pp. 728-33.
Sabrosky, C. W. Family Chloropidae. pp. 773-93.
Sabrosky, C. W. Family Odiniidae. pp. 793-94.
Sabrosky, C. W. Family Anthomyzidae. pp. 819-20.
Sabrosky, C. W. Family Asteiidae. pp. 823-25.
Sabrosky, C. W., and P. H. Arnaud, Jr. Family Tachinidae. pp. 961-1108.
Steyskal, G. C. Family Micropezidae. pp. 633-36.
Steyskal, G. C. Family Neriidae. p. 637.
Steyskal, G. C. Family Tanypezidae. p. 641.
Steyskal, G. C. Family Richardiidae. pp. 641-42.
Steyskal, G. C. Family Otitidae. pp. 642-54.
Steyskal, G. C. Family Platystomatidae. pp. 655-57.
Steyskal, G. C. Family Pyrgotidae. pp. 657-58.
Steyskal, G. C. Family Helcomyzidae. pp. 678-79.
Steyskal, G. C. Family Dryomyzidae. pp. 680-81.
Steyskal, G. C. Family Sepsidae. pp. 681-85.
Steyskal, G. C. Family Sciomyzidae. pp. 685-95.

A Catalog of the Diptera of America North of Mexico. 1965 USDA, Agr. Handbook 276. (continued)
Steyskal, G. C. Family Piophilidae. pp. 710-13.
Steyskal, G. C. Family Thyreophoridae. pp. 713-14.
Steyskal, G. C. Family Clusiidae. pp. 805-07.
Steyskal, G. C. Family Acartophthalmidae. p. 808.
Stone, Alan. Family Blephariceridae. pp. 98-100
Stone, Alan. Family Culicidae. pp. 105-20.
Stone, Alan. Family Thaumaleidae. p. 120.
Stone, Alan. Family Simuliidae. pp. 181-89.
Stone, Alan. Family Braulidae. p. 726.
Stone, Alan, and Jean Laffoon. Family Sciaridae. pp. 229-36.
Stone, Alan, C. W. Sabrosky, W. W. Wirth, R. H. Foote, and J. R. Coulson (editors). 1696 pp.
Wirth, W. W. Family Ceratopogonidae. pp. 121-42.
Wirth, W. W. Family Lonchopteridae. pp. 530-31.
Wirth, W. W. Family Canaceidae. pp. 733-34.
Wirth, W. W. Family Ephydridae. pp. 734-59.
Wirth, W. W. Family Curtonotidae. p. 759.
Wirth, W. W. Family Aulacigastridae. p. 823.
Wirth, W. W., Y. S. Sedman, and H. V. Weems. Family Syrphidae. pp. 557-625.

A Catalog of the Diptera of the Americas South of the United States. 1966. Dep. Zool., Sec. Agr. (Sao Paulo).
Sabrosky, C. W. Preface, No. 1. pp. i-ii.
Sabrosky, C. W. Family Asteiidae, No. 92. 5 pp.
Stone, Alan. Family Dixidae, No. 10. 4 pp.
Stone, Alan. Family Chaoboridae, No. 11. 8 pp.
Stone, Alan. Family Thaumaleidae, No. 13. 2 pp.
Stone, Alan. Family Braulidae, No. 73. 1 p.

Baker, E. W. 1965. A review of the genera of the family Tydeidae (Acarina). Advan. Acarology. 2:95-133, illus.

Herring, J. L. 1966. The Anthocoridae of the Galapagos and Cocos Islands (Hemiptera). Proc. Entomol. Soc. Wash. 68:127-30, illus.

Marsh, P. M. 1965. The Nearctic Doryctinae, I. A review of the subfamily with a taxonomic revision of the tribe Hecabolini (Hymenoptera: Braconidae). Ann. Entomol. Soc. Amer. 58:668-99, illus.

Russell, L. M. 1966. Aphid pests of corn in the United States. Proc. North Cent. Br. Entomol. Soc. Amer. 20:51.

Steyskal, G. C. 1965. The family Tanypezidae in North America (Diptera, Acalyptratae). Proc. Entomol. Soc. Wash. 67:201.

Stone, Alan, J. E. Scanlon, D. L. Bailey, M. D. Delfinado, and R. A. Bram. 1966. Preliminary Keys to the Mosquitoes of Vietnam. First Revision. Separate publication, offset, U. S. Army. 76 pp.

General.

Hoenigsberg, H. F., and Y. G. de Navas. 1965. Population genetics in the American tropics. I. Concealed recessives in different bioclimatic regions. Evolution 19:506-13. (P. L. 480)

Sabrosky, C. W. 1965. Request for a declaration against the suppression of nomina dubia. Bull. Zool. Nomenclature. 22:265-66.

Sabrosky, C. W. 1966. Opposition to the proposed designation of a type-species for Phasia Latreille. Bull. Zool. Nomenclature. 23:9-11.

Sabrosky, C. W., and E. C. Zimmerman. 1966. Trypetesinae and Trypetesini (Lacordaire): Proposed emendation of family-group names under the plenary powers (Insecta, Coleoptera). Bull. Zool. Nomenclature. 23:46-47.

Sabrosky, C. W. 1966. Priority and Recognition. Science 151:1479-80.

Steyskal, G. C. 1965. Notes on uninominal nomenclature. Syst. Zool. 14:346-48.

Steyskal, G. C. 1965. Trend curves of the rate of species description in zoology. Science. 149:880-82, illus.

PUBLICATIONS -- STATE EXPERIMENT STATIONS AND COOPERATIVE PROGRAMS

Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites, and Ticks.

Beardsley, J. W., Jr. 1965. The male of Antonina crawii (Homoptera: Pseudococcidae). Proc. Hawaii Entomol. Soc. 19(1):47-49. (Hawaii)

Beardsley, J. W., Jr. 1965. A new armored scale insect from Araucaria (Homoptera: Diaspididae). Proc. Hawaii Entomol. Soc. 19(1):51-54. (Hawaii)

Carayon, J., and R. L. Usinger. 1965. A new genus of Neotropical Anthocoridae that resembles the bed bug. Pan-Pacific Entomol. 41:200-204. (Calif.)

Chant, D. A. 1965. Generic concepts in the family Phytoseiidae (Acarina: Mesostigmata). Canada Entomol. 97(4):351-74. (Calif.)

Chant, D. A., and Evert E. Lindquist. 1965. Nabiseius duplicisetus, a new genus and species of Otopheidomeninae (Acarina: Phytoseiidae) from nabid bugs. Canada Entomol. 97(5):515-21. (Calif.)

Chemsak, J. A. 1965. A new subspecies of Oeme costata LeConte, with observations on the habits of larvae and adults. J. Kansas Entomol. Soc. 38:351-55. (Calif.)

Chemsak, J. A., and E. G. Linsley. 1965. The elaphidionine subgenus Protaneflus. Proc. Entomol. Soc. Wash. 67:24-28. (Calif.)

Chemsak, J. A., and E. G. Linsley. 1965. New genera and species of North American Cerambycidae. Pan-Pacific Entomol. 41:141-53. (Calif.)

Krantz, G. W. 1965. A new species of Macrochelidae associated with bark beetles of the genera Ips and Dendroctonus. Proc. Kansas Entomol. Soc. 38:145-53. (Oreg.)

La Berge, Wallace E., and Paul D. Hurd, Jr. 1965. A new subgenus and species of matinal Andrena from the flowers of Sicyos (Cucurbitaceae) in Mexico (Hymenoptera: Apoidea). Pan-Pacific Entomol. 41:186-93. (Nebr.)

Powell, J. A. 1965. Supplementary notes on North American and Mexican species of Decodes and Argyrotaenia, with description of new species (Lepidoptera: Tortricidae). Proc. Biol. Soc. Wash. 78:65-80. (Calif.)

Smith, Clyde F. 1965. Tiliphagus lycoposugus, new genus, new species (Aphididae: Homoptera) from Tilia americana and Lycopus virginicus. Ann. Entomol. Soc. Amer. 58:781-86. (N. C.)

Usinger, R. L., and Norihiro Ueshima. 1965. New species of bat bugs of the Cimex pilosellus complex. Pan-Pacific Entomol. 41:114-17. (Calif.)

Identification of Insects, Mites, and Ticks.

Bohart, R. M. 1965. Synonymy of North American Odynerini described by Peter Cameron. Pan-Pacific Entomol. 41:107-13. (Calif.)

Comegys, George R., and John E. Schmitt. 1965. A list of the Thysanoptera or thrips of New Jersey. J. New York Entomol. Soc. 73:28. (N. J.)

Cunningham, Hugh B., and Herbert H. Ross. 1965. Characters for specific identification of females in the leafhopper genus Empoasca (Hemiptera: Cicadellidae). Ann. Entomol. Soc. Amer. 58:620-23. (Ill.)

Marston, Norman. 1965. Some recent modifications in the design of Malaise insect traps with a summary of the insects represented in collections. J. Kans. Entomol. Soc. 38:154-62. (Kans.)

Young, D. A. 1965. Some Cicadelline lectotypes of Breddin species. Beit. Entomol. 15:11-14. (N. C.)

Young, D. A. 1965. Notes on Fabrician species of Cicadellinae in Copenhagen and Kiel with lectotype designations. Entomol. Medd. 34:10-18 (N. C.)

Young, D. A. 1965. Cicadelline types in the British Museum. Bull. Brit. Mus. 17:161-99. (N. C.)

Preparation of Keys and Monographs on the Classification, Distribution, Morphology, and Biology of Insects and Related Arthropods.

Bohart, R. M., and L. A. Stange. 1965. A revision of the genus Zethus Fabricius in the Western Hemisphere. Univ. Calif. Pub. Entomol. 40:1-208. (Calif.)

Chant, D. A. 1965. The identity and distribution of species of Phytoseius (Acarina: Phytoseiidae) in Canada. Canada Entomol. 97(9):897-909. (Calif.)

Chemsak, J. A., and E. G. Linsley. 1965. A revised key to the species of Elytroleptus with notes on variation and geographical distribution. Pan-Pacific Entomol. 41:193-99. (Calif.)

Hardy, D. Elmo, et. al. 1965. Insects of Hawaii, Vol. 12, Diptera: Cyclorrhapha, family Drosophilidae. Univ. Hawaii Press. 814 pp. (Hawaii)

Knutson, L. V., and L. Lyneborg. 1965. Danish acalyptrate flies. 3. Sciomyzidae (Diptera). Entomol. Medd. 34:61-101. (N. Y.)

Krantz, G. W. 1965. A review of the genus Neopodocinum Oudemans 1902. (Acarina: Macrochelidae). Acarologia 7(2):139-226. (Oreg.)

Laffoon, Jean L. 1965. Family Mycetophilidae (Fungivoridae). pp. 196-229. in: Alan Stone, et. al. (editors). A Catalog of the Diptera of America North of Mexico. USDA, Agr. Res. Serv., Agr. Handbook 276. (Iowa)

Metcalf, Z. P. 1965. General Catalogue of the Homoptera. Fascicle VI. Cicadelloidea. Part 1, Tettigellidae. Agr. Res. Serv. USDA. 730 pp. (N. C.)

Metcalf, Z. P., and V. Wade. 1965. Fascicle I, Supplement, Membracoidea. 2 Sections. N. C. State Univ. 1560 pp. (N. C.)

O'Brien, Lois Breimeier, and Paul D. Hurd, Jr. 1965. Carpenter bees of the Subgenus Notoxylocopa (Hymenoptera: Apoidea). Ann. Entomol. Soc. Amer. 58:175-96. (Calif.)

Painter, Reginald H., and Elizabeth M. Painter. 1965. Family Bombyliidae. pp.407-46. in: A Catalog of the Diptera of America North of Mexico. USDA. Agr. Handbook 276. (Kans.)

Reinhard, H. J. 1965. Review of the miltogrammid genus Eumacronychia (Sarcophagidae: Diptera). Canad. Entomol. 97:337-50. (Tex.)

Schlinger, E. I. 1965. In A Catalog of the Diptera of America North of Mexico by Stone, Alan, et. al. (editors). USDA, Agr. Res. Serv.,

Wash., D. C. pp. I-1696. (Acroceridae, pp. 403-07). (Calif.)

Young, D. A. 1965. Western Hemisphere Mileewanini. Zool. Beltrage (N.F.) 11:369-80. (N. C.)

General.

Ross, Herbert H., George C. Decker, and Hugh B. Cunningham. 1965. Adaptation and differentiation of temperature phylogenetic lines from tropical ancestors in Empoasca. Evolution 18(4):639-51. (Ill.)

AREA NO. 18. FOREIGN EXPLORATION, INTRODUCTION AND EVALUATION OF BIOLOGICAL CONTROL AGENTS

Problem. Many of the most serious insect and weed pests in the United States have been accidentally introduced from foreign countries without the insect enemies that keep them under control in their native homes. Some of the harmful insects so introduced have been effectively controlled by later introduction of their parasites and predators. Foreign exploration for beneficial biological control agents of insects and their subsequent introduction, colonization, and evaluation in this country is now a well established practice in the control of introduced insect pests. The use of imported insects to control introduced noxious weeds, although a more recent practice, has shown much promise. The biological approach to the control of insect and weed pests has great potential. Therefore, further foreign exploration is needed and additional research is necessary on the biology, ecology, nutritional requirements, and the most effective manner of utilizing natural control agents if they are to be used to maximum advantage. There is much concern by the public over the insecticide and other residue problems in foods and by conservationists over the potential hazards of insect-control chemicals to fish and wildlife. More effective use of natural control agents in meeting destructive insect and noxious weed problems could materially contribute to the ultimate objective of overcoming the pesticide residue and other hazard problems associated with the use of chemicals for the control of insects and weeds.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program on the use of beneficial insects. Basic and applied research is conducted on insect parasites and predators of insect pests and on insects that attack weeds, including foreign explorations for beneficial species and their introduction, liberation, and evaluation in this country. A laboratory is maintained at Nanterre (near Paris), France, for studies on the parasites and predators of agricultural pests that have accidentally been introduced from Europe into the United States. At a station in Rome, Italy, studies are in progress on insects attacking a number of weeds, including Scotch broom, tansy ragwort, Dalmation toadflax, Mediterranean sage, Russian knapweed, and halogeton. Research supported by the U. S. Army Corps of Engineers on insects affecting aquatic weeds, especially alligatorweed and waterhyacinth, is being conducted at the National Agricultural Research Center in Castelar (near Buenos Aires), Argentina. In the United States, a receiving station and laboratory is maintained at Moorestown, New Jersey, where major emphasis is given to receiving, propagating, and transshipping insect parasites to appropriate liberation points. A laboratory for receiving, studying, and liberating insects affecting range weeds is located at Albany, California. Studies regarding entomophagous insects are also conducted at Riverside, California. The work at Albany

and Riverside is conducted with the cooperation of the University of California and the California Experiment Stations. A long-range program including numerous aspects of biological control of insect pests is continuing at Columbia, Missouri, conducted in cooperation with the University of Missouri and the Missouri Agricultural Experiment Station. Seven grants and four contracts have been executed that are concerned with the study of insect parasites and predators. The grants are to Washington State University, the University of Missouri, the University of Arkansas, Louisiana State University, the University of Minnesota, Cornell University, and the University of Connecticut. The contracts are with the University of Idaho, the University of Indiana, the University of California at Riverside, and the California Academy of Sciences in San Francisco.

The Federal scientific effort devoted to research in this area totals 23.0 scientist man-years. Of this total, 3.0are devoted to search for and importation of foreign parasites and predators of insect pests; 2.6 to search for and importation of foreign insect enemies of weeds; 11.0 to basic biology, physiology, nutrition, and evaluation; 5.4 to receipt, liberation, and establishment of foreign insect enemies of insect pests and weeds; and 1.0 to program leadership.

In addition, Federal support of research in this area conducted under grants and contracts provides for a total of 4.0 scientist man-years. Of this total, 0.9 is devoted to search for and importation of foreign parasites and predators of insect pests; 2.5 to basic biology, physiology, nutrition, and evaluation; and 0.6 to studies of native insects that attack weeds of foreign origin.

Twenty grants from P.L. 480 funds providing for 49 scientist man-years have been executed for projects directly concerned with the study of insect parasites and predators. Eleven of these projects involve exploration for beneficial species that might be shipped to the United States for trial and release against agricultural pests here.

Grants for five P.L. 480 projects providing for 12 scientist man-years have been executed for studies on the biological control of weeds.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 11.6 professional man-years is devoted to this area of research.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Search for and Importation of Foreign Parasites and Predators of Insect Pests.

1. Parasites and Predators. Twenty-one species of parasites or predators of seven kinds of insect pests were collected in Europe for introduction into the United States, and six species of parasites or predators of four

kinds of insect pests were sent to the United States from P.L. 480 projects in India. All of these beneficial insects, approximately 32,000 specimens in 116 shipments, were received by the Moorestown, New Jersey, laboratory for screening, testing, and transshipment of living material to liberation points throughout the United States or to Department field stations or State Experiment Stations for further testing and propagation before liberation. Twenty-nine beneficial species were collected locally or reared from laboratory stocks at Moorestown and 179 shipments (almost 45,000 specimens) made available to State or Federal laboratories for release and future evaluation. Shipments of beneficial insects were sent to 22 States.

2. Brazilian Plant-feeding Insects and their Parasites and Predators. By means of a P.L. 480 grant, a bibliography of references to Brazilian plant-feeding insects and their parasites and predators has gone to press. The bibliography is to be a companion volume to a catalog on the same subject which is in its final stages of preparation.

3. Fall Armyworm and Potato Leafhopper. Eleven kinds of parasitic insects, a nematode, and two insect diseases were reared from or observed to attack larvae of the fall armyworm in Uruguay during the course of a P.L. 480 project in that country that expired during the fiscal year. Four of these parasites appear to be new to science and are potentially useful as biological agents that may reduce damaging populations of the fall armyworm in the United States.

Exhaustive searches in Uruguay during the course of the same project revealed the presence of three hemipterous predators and two wasp parasites that may have promise in controlling economically important outbreaks of the potato leafhopper in the United States.

A P.L. 480 project in India is effectively studying the kinds of parasites, predators, and diseases of a number of economically important insects there.

4. Enemies of Aphids. A research program in Taiwan, supported by P.L. 480 program funds, is charged with a search for parasites, predators, and microorganisms attacking aphids infesting citrus, tobacco, and vegetables. Researchers have already discovered a surprisingly large number of natural enemies of aphids in that country, and they are investigating the biologies of some of the ones that seem to have the most promise for introduction into the United States. In another P.L. 480 project, a general survey of aphids and their natural enemies occurring in India continues to demonstrate the large number of aphid species occurring there and the numerous interesting parasite species that have promise for eventual aphid control.

5. Coconut Rhinoceros Beetle. Recent explorations for natural enemies of this serious pest of coconuts, supported by a P.L. 480 grant, have uncovered what appear to be promising control agents. These agents are an unidentified bacterium and a nematode, both attacking the larvae of the beetle. These organisms were found in rhinoceros beetle larvae collected on the Laccadive

Islands off the west coast of India. Larvae are killed when thick suspensions of the nematode are administered to them.

B. Search for and Importation of Foreign Insect Enemies of Weeds.

1. Canada Thistle. This is one of the most important weed pests in the United States and is a serious problem in pastureland, rangeland, and agronomic crops in many areas in the northern part of the country and in Canada. A flea beetle, Haltica carduorum, attacks this thistle in Europe. The insect was released in Canada after adequate tests proved it would complete its development on the thistle only. Stock of the beetle was studied for approximately two years in quarantine at Albany, California, and tests run on additional plants of agricultural or ornamental importance. Satisfactory proof of the beetle's specificity was gathered; and sexually mature, mating adults were released at 10 locations in four States in April, May, and June 1966. Proof of establishment has not been reported.

2. Tansy Ragwort. A seed-head fly, Hylemya seneciella, attacks this weed in Europe. Larvae of the fly attack and destroy developing seeds and reduce rate of spread of the weed. Proof that the insect will develop on ragwort only was obtained through adequate research in Europe. Approximately 16,000 puparia of the fly were gathered in the vicinity of Paris, France, in 1965 and shipped to Albany, California. Adult flies from these puparia were released in June 1966. More than 2,200 flies were released at Fort Bragg, California, and approximately 2,000 released in Oregon.

3. Natural Enemies of Eurasian Watermilfoil. A project in Pakistan, under the P.L. 480 program, is designed to determine whether any insects exist in that country that could be important in the control of Eurasian watermilfoil, a very serious weed pest in several eastern States. Observations have been made on a number of insects attacking that plant, among which is a weevil that feeds on the plant well below the water surface. The weevil's biology and adaptations to living in the water are being investigated.

4. Enemies of Witchweed and Waterhyacinth. In a P.L. 480 project in India, a survey of these two plants has been underway for some time. As species are located in various areas under consideration, their habits and biologies are studied to determine to what extent they may be of value in the control of these two important weeds.

C. Basic Biology, Physiology, Nutrition, and Evaluation.

1. Green Peach Aphid. The green peach aphid has a number of common parasites in Missouri, but in the Southeastern United States, the usual parasites of this aphid on tobacco seem to be absent. When greenhouse aphid colonies in Missouri were found to support large numbers of Aphidius matricariae, a parasite not usually found associated with the green peach aphid, rearing of the parasite was initiated in the laboratory for possible introduction into Florida tobacco growing areas. Preliminary observations on rearing indicate

that the parasite may have the potential of reducing populations of the aphid below economically important levels.

2. Cutworms. A mermithid nematode belonging to the genus Hexamermis has been reared from two cutworm species in Missouri. Adult nematodes presumed to be the same species have been found in soil samples taken for the purpose of assaying the distribution of this potentially important parasite. Extensive examinations of cutworms had previously revealed little parasitization by Hexamermis, but insect parasites were found to be common.

Investigations are also being conducted on the biology and habits of several insect parasites of cutworms that appear to have promise in the control of these pests.

3. Cabbage Looper. In the course of explorations under P.L. 480 programs for parasites of corn borers in India, shipments of a species of Pimpla (Ichneumonidae) were sent to the United States in 1965 to control the European corn borer. The latter was not accepted readily as a host by the parasite, but pupae of the cabbage looper gave satisfactory returns of progeny and a favorable sex ratio. The parasite is a vigorous species and might well be valuable as a parasite of the cabbage looper.

4. Alfalfa Weevil. Larvae of the alfalfa weevil collected near Svalor, Sweden, and in other European localities have exhibited a disease that causes a cessation of feeding followed by a change in the color and turgidity of the body. The disease during previous years has reached epidemic proportions in larvae being held for parasite emergence. Preliminary transmission studies have been negative, but additional experiments are planned.

5. Insects to Control Alligatorweed. Studies in Argentina on the biology of the alligatorweed thrips, which is being investigated for introduction into the United States as a possible control agent for that weed, show the mechanisms by which it is able to expand its range. This insect shows considerable promise, as it appears to be restricted in its host preferences and to be adaptable to North American climates where alligatorweed is a problem.

Field observations of Vogtia malloi, a phycitid moth found to attack the stems of alligatorweed in Argentina, are being made to determine its life history and ecology. Host-plant cultures are being prepared to facilitate laboratory rearing of this insect for studies of its host specificity.

A survey of insects naturally occurring on alligatorweed in Louisiana is underway to determine if any South American species may have been imported with the weed. A number of species has been identified, but none so far has shown promise in the control of that weed; and none of foreign origin has been discovered.

6. Tansy Ragwort. Field investigations have revealed the presence in large numbers of a gall midge, Contarinia jacobaeae (Loew), in the flowers of tansy ragwort growing in the vicinity of Paris, France. The midge has been reported causing a great deal of damage to ragwort blossoms in England and in Switzerland, and its presence in large numbers indicates it may be a candidate for further study in the control of the weed.

7. Face Flies. A laboratory culture of face flies at Columbia, Missouri, was found to be infested with a species of nematode known previously only from New York State. The rather complicated life cycle of this nematode was worked out to determine which of its developmental stages might be responsible for any reductions in face fly populations, and studies are continuing to assess the nematode's possible economic importance.

8. Corn Rootworm. Relatively high percentages of the southern corn rootworm in Missouri were found to be parasitized by a tachinid fly, probably Celatoria sp. Contrasting low percentages of parasitization by this fly of the northern corn rootworm indicate that the parasite survives more successfully in the southern populations of rootworms that have more than one generation per year.

9. Clover Root Curculio. Small nematodes, as yet unidentified, have been found in the dissection of about 700 larvae of this beetle. Some of these parasites have appeared to be pathogenic, and further studies are being conducted to determine their actual importance.

10. Tachinid Flies. Under a grant to Washington State University, field studies on a number of tachinid flies and their hosts are being made. So far, species of more than 10 fly genera have been studied, and their biological relationships to their hosts are now being investigated. A large number of tachinid parasites have been reared from tent caterpillars on San Juan Island in Puget Sound.

11. Testing of Chemical Stimuli. A number of fatty acids and protein hydrolysates are being tested at the University of Minnesota under a research grant to determine their attractiveness to coccinellid beetles. Early field studies show that corn of a certain genetic stock is more attractive to coccinellids than others and that there might be an intra-specific attraction among the beetles themselves, possibly of a pheromone nature. These relationships are being tested under laboratory conditions, and their causes are being investigated.

12. Parasitic Beetles. Research on parasitic carabid beetles is being conducted at the University of Arkansas under a research grant. Preliminary observations indicate that the host of the parasitic beetle, Lebia analis, is the chrysomelid beetle, Disonycha glabrata, an insect associated with plants of the genus Amaranthus. Three other species of Lebia are known to attack chrysomelids in Arkansas, each parasitic species being associated with a single kind of host.

13. Waterhyacinth. In another grant study at Louisiana State University, aquatic weeds are being surveyed for possible agents in their control. A sizeable infestation of spider mite was found to be causing discoloration of the plants, although no reduction in plant size or number was evident. This situation is being watched closely for future possible developments.

14. Rangeland Weeds. A grant to the University of Idaho provides for a study of the insects that feed on rangeland weeds of foreign origin in that State. A survey now underway is adding materially to our knowledge of the distribution of such weeds and the native insects attacking them. Preliminary studies have been undertaken on the weevil, Cosmobaris americana, which has been commonly found associated with two of the introduced rangeland weeds concerned in the project.

15. Scale Insects on Citrus. A study of the biology of citrus scale insects in Israel has been initiated as a P.L. 480 grant. Basic work on a number of species that are potentially important in scale control is underway, and manuscripts dealing with parasite and predator identification and occurrence have already been submitted for publication.

16. Insects on Rice. Data from continuing research on rice insects and their parasites in Pakistan, made available by means of a P.L. 480 grant, indicate that some rice varieties have apparently gained resistance to lepidopterous borer attack. The development of resistant rice varieties may be an important natural control factor and is being given emphasis.

17. Aphids. Detailed observations on the biology of Coccinella septempunctata are being made under P.L. 480 program sponsorship in the vicinity of Hyderabad, India. The studies include rearing this coccinellid in the laboratory to determine the effect of various natural diets on longevity, oviposition, and hibernation and observing the beetle's behavior under simulated field conditions. It is hoped that light can be shed on the reasons for this beetle being the foremost aphid enemy in India, thus giving an idea as to how the beetle may be established successfully in the United States.

18. Mass Rearing of Parasites. Although large numbers of individual parasites have not been produced, promising methods for mass rearing both parasites and hosts are being developed in a P.L. 480 program in India. Larvae of Heliothis, the principal host in this project, support a large number of parasite species in India. During the course of the work, an outbreak of nuclear polyhedrosis virus reduced host colonies considerably, providing further study material of a disease that is of interest because it commonly causes high mortality in Heliothis larvae.

19. Sugarcane Borers. A P.L. 480 project has given a large amount of information on a number of sugarcane borers at various locations in India and on the incidence of parasites associated with the borers. A method has also been developed for the large-scale rearing of Campyloneurus mutator, a parasite that may have some potential in the control of the rice stalk borer

in the United States.

20. Corn Borers. Continued exhaustive surveys in Pakistan through P.L. 480 support failed to uncover borers of corn in addition to those discovered in previous years. Parasites of the known borers are being studied, the most widely distributed parasite being Glyptomorpha deesae. A species of Ostrinia different from the European corn borer is found in a thistle, Cnicus wallichii, only, not in corn. Parasitism of this borer is consistently at a low level.

D. Receipt, Liberation, and Establishment of Foreign Insect Enemies of Insect Pests and Weeds.

1. Gypsy Moth. During the period 1900-1935, very large numbers of Brachymeria intermedia, a Palearctic parasite of the gypsy moth, were released in New England, apparently without success. However, the release in 1963 and 1964 of almost 17,000 specimens of this parasite in Connecticut and New Jersey appears to have resulted in successful establishment since two lots of field-recovered specimens, both from different localities in Connecticut, have been identified. Existence of a specimen collected in 1941 may indicate that species was established by early releases but persisted in low numbers.

2. Pea Aphid. In the Southern States, the introduced parasite, Aphidius smithi, has become the dominant species in the early-season control of the pea aphid. Moreover, during mid-June, pea aphid collections in eastern Ohio, northern New Jersey, and southern New York for the first time yielded high percentages of the parasite, indicating that it is able to withstand low winter temperatures and that it is becoming an important factor in pea aphid control in the Eastern United States also.

3. Alligatorweed. At a one-fifth-acre site near Jacksonville, Florida, where the alligatorweed flea beetle was released in 1965, there has been a remarkable expansion of the population of that insect. The weed on the site has been completely defoliated, leaving only badly chewed, bare stems. At the end of May, the beetles had moved more than a mile up and down the Ortega River from the release site.

4. Cereal Leaf Beetle Parasites. The mymarid egg parasite, Anaphes sp., of the cereal leaf beetle is being reared in Indiana as well as in Michigan where it is in the 25th generation from material originally obtained in Europe. Releases were made during late May and June in Indiana and Michigan. Parasitized host eggs were recovered at the release sites.

5. Smaller European Elm Bark Beetle Vector of Dutch Elm Disease. The ichneumonid parasite Dendrosoter protuberans was obtained in France and shipped to the Northeastern Forest Experiment Station at Delaware, Ohio, where it was successfully colonized and has since been released in Ohio, Michigan, and Missouri.

PUBLICATIONS--USDA AND COOPERATIVE PROGRAM

Search for and Importation of Foreign Parasites and Predators of Insect Pests.

Ahmad, Rafiq, and M. A. Ghani. 1966. A new species of Chilocorini (Coleoptera: Coccinellidae) from Pakistan. Proc. Roy. Entomol. Soc. London. Ser. B. 35:9-10. (P.L. 480, Pakistan)

Chapin, E. A., and Rafiq Ahmad. 1966. A new species of Coccinellid feeding on scale insects in West Pakistan. Entomophaga, 11:213-15. (P.L. 480, Pakistan)

Mathur, S. B., and R. P. Srivistava. 1966. Study of entomogenous fungus Entomophthora aphidis Hoffman, parasitic on the spotted alfalfa aphid. Indian J. Entomol. 28:119-20, illus. (P.L. 480, India)

Paschke, J. D. 1965. Infection of the cereal leaf beetle, Oulema melanopa (L.) by Beauveria bassiana (Balsamo) Vuillemin. J. Invert. Pathol. 7:101-102. (Contract, Purdue University)

Srivistava, R. P., S. B. Mathur, and M. L. Sahani. 1965. An Entomophagous fungus, Entomophthora sp., pathogenic on Empoasca devastans Distant, a Bhindi jassid. Indian J. Entomol. 27:367-68. (P.L. 480, India)

Subba Rao, B. R. 1965. A key to species of Anicetus Howard, 1896, and Descriptions of a new species from India. Proc. Roy. Entomol. Soc. London (B) 34(5-6):71-75, illus. (P.L. 480, India)

Subba Rao, B. R. 1965. A new genus of Encyrtidae from India. Proc. Roy. Entomol. Soc. London (B) 34(11-12):150-52, illus. (P.L. 480, India)

Subba Rao, B. R., G. G. Kundu, V. K. Sharma, R. K. Anand, and Samarit Rai. 1965. New records of parasites of the groundnut leaf miner, Stomopteryx nertaria (Meyrick). Indian J. Entomol. 27:355-57. (P.L. 480, India)

Search for and Importation of Foreign Insect Enemies of Weeds.

Rag Naj, T. R. 1965. Thread blight of waterhyacinth. Current Sci. 34(21):618-19, illus. (P.L. 480, India)

Basic Biology, Physiology, Nutrition, and Evaluation.

Andres, L. A. 1966. Host specificity studies on Phyrdiuchus topiarius and Phyrdiuchus sp. J. Econ. Entomol. 59:69-76, illus.

Angalet, G. W., and L. A. Andres. 1965. Parasites of two weevils, Microlarinus lareynii and Microlarinus lypriformis, that feed on puncture vine, Tribulus terrestris. J. Econ. Entomol. 58:1167-68.

Dowden, P. B. 1965. Controlling forest insects by natural and biological means. In Insects in Southern Forests, C. B. Marlin, Editor, Louisiana State University Press, Baton Rouge, La. pp. 64-72.

Drea, J. J., and T. B. O'Connell. 1965. Life history and host specificity studies of Heterographis fulvobasella Ragonot. J. Econ. Entomol. 58:1110-1115, illus.

Dysart, R. J. 1966. Natural enemies of the banded-wing whitefly, Trialeurodes abutilonea. Ann. Entomol. Soc. Amer. 59:28-33.

Puttler, Benjamin. 1966. Notes on two parasites attacking a Lema sp. (Coleoptera: Chrysomelidae). J. Econ. Entomol. 59:475-76.

Streams, F. A., and R. W. Fuester. 1966. Patasson luna in overwintering eggs of the alfalfa weevil. J. Econ. Entomol. 59:331-33.

Receipt, Liberation, and Establishment of Foreign Insect Enemies of Insect Pests and Weeds.

Angalet, G. W., and L. W. Coles. 1966. The establishment of Aphidius smithi in the Eastern United States. J. Econ. Entomol. 59:769-70, illus.

Dysart, R. J., and Benjamin Puttler. 1965. The alfalfa weevil parasite Bathyplectes curculionis in Illinois and notes on its dispersal. J. Econ. Entomol. 58:1154-55.

Puttler, Benjamin. 1966. Biological notes on some hyperparasites of Bathyplectes curculionis (Thomson). J. Econ. Entomol. 59:483-84.

PUBLICATIONS--STATE EXPERIMENT STATIONS AND COOPERATIVE PROGRAMS

Butler, G. D., Jr. 1965. Spanogonicus albofasciatus as an insect and mite predator. (Hemiptera: Miridae). J. Kans. Entomol. Soc. 38:70-75. (Ariz.)

Butler, G. D., Jr., and F. G. Werner. 1965. Light-trap records of three cactus-eating moths in Arizona. Pan-Pacific Entomol. 41:10-12. (Ariz.)

DeBach, Paul. 1965. Weather and the success of parasites in population regulation. Canad. Entomol. 97(8):848-63. (Calif.)

DeBach, Paul. 1965. Some biological and ecological phenomena associated with colonizing entomophagous species. In The genetics of colonizing species. Academic Press. N.Y. 287-306. (Calif.)

Knutson, L. V., J. W. Stephenson, and C. O. Berg. 1965. Biology of a slug-killing fly, Tetanocera elata (Diptera: Sciomyzidae). Proc. Malac. Soc. Lond. 36:213-20. (N.Y.)

Legner, E. F.,and E. C. Bay. 1965. Culture of Hippelates pusio (Diptera: Chloropidae) in the West Indies for natural enemy exploration and some notes on behavior and distribution. Ann. Entomol. Soc. Amer. 58:436-40. (Calif.)

Legner, E. F., E. C. Bay,and C. W. McCoy. 1965. Parasitic natural regulatory agents attacking Musca domestica in Puerto Rico. J. Agr. Univ. Puerto Rico 49(3):368-76. (Calif.)

Legner, E. F.,and E. C. Bay. 1965. La primera exploration para buscar enemigos naturales de Hippelates en Puerto Rico (Insects: Chloropidae). Carib. J. Sci. 4(4):467-71. (Calif.)

Pimentel, D.,and R. Al-Hafidh. 1965. Ecological control of a parasite population by genetic evolution in the parasite-host system. Ann. Entomol. Soc. of Amer. 58:1-6. (N.Y.)

AREA NO. 19. INSECT PATHOLOGY

Problem. Basic investigations on viruses, fungi, bacteria, nematodes and protozoa are needed to fully exploit the use of such microorganisms as an approach to insect control. There is much interest in the use of these natural insect-control agents to overcome the growing concern over chemical residues following the application of insecticides to agricultural crops and livestock, and the increasing resistance of some insects to certain insecticides. The utilization of pathogens to produce diseases in insect populations, and so reduce them and the damage they cause, is an approach that has already shown great promise. Microorganisms that are pathogenic for insects are generally very efficient when used properly. They are specific for their insect hosts and harmless to men and other vertebrates. Basic research is needed for a thorough understanding of insect pathogens, including their growth and nutritional requirements, their resistance to environmental factors, and their mutability and mode of action, both in the laboratory and the field. Such knowledge must be obtained before these organisms can be used effectively in the control of insect pests.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing basic research program on the growth, nutritional requirements and mode of action of viruses, bacteria, and nematodes affecting insects. At the Pioneering Research Laboratory on Insect Pathology at Beltsville, Md., studies are in progress on mutability-induced changes in virulence of insect diseases, and the resistance of insects to diseases, including studies of the effect of the environment on the pathogens. A comprehensive reprint library on insect pathology is being assembled. Collections of all spore formers and viruses known to cause disease in insects are being obtained from world-wide contributors. A service involving the diagnosis of unhealthy insects is now available to Division, State, and University laboratories.

Two new lives of research have been started within the past year. A laboratory for serological investigations to aid in all aspects of the research conducted at Beltsville and an insect tissue culture laboratory to investigate production of insect tissue and to study the invasion metabolism and production of insect viruses.

The program includes collaborative studies with the Pesticide Chemicals Research Branch on instrumentation for monitoring insect activity, internal temperatures of insects, and effect of gaseous atmosphere on metabolism and development of insects. Collaborative studies are also under way with the Pioneering Research Laboratory on Insect Physiology on the effect of microorganisms on insect sterol requirements. A cooperative project to study the progress of the non-inclusion virus disease of the citrus red mite, through electron microscopy, has been set up with the Fruit and Vegetable

Insects Research Branch. A second cooperative study on the serology of this virus is under way in cooperation with the Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada.

Contracted research continued with Rosner-Hixson Laboratories for studies of mammalian toxicity and pathogenicity of insect viruses and with Ohio State University, Rutgers University and University of Maryland for research on the production of virus diseases of four major insect pests. A contract was negotiated with Nutrilite Products Inc. to study the production of viruses pathogenic for two more insects of major economic importance and to investigate the possibility of selecting more virulent viral strains of the nuclear polyhedrosis of the cotton bollworm, Heliothis zea. A contract was negotiated with Midwest Research Institute to attempt to propagate an insect virus in bacteria and another contract was negotiated with Cornell University, Geneva to determine the relative virulence of two, coexisting viral pathogens of the cabbage looper.

A cooperative project has been conducted with the Fruit and Vegetable Insects Research Branch to study the serology of the non-inclusion virus of citrus red mite, in order to develop a tool for diagnosis of the disease. Cooperative studies on the identification of B. thuringiensis varieties are being carried out with the Institute of Pasteur, Paris, France.

Federal Scientific effort devoted to research in this area totals 10.0 scientist man years. Of this number 4.4 is devoted to virus diseases of insects; 4.6 to bacterial, nematode, protozoan and fungal diseases of insects, and 1 to the discovery and study of new pathogens of insects.

In addition Federal support for 3.3 professional man-years devoted to research on virus disease of insects is provided under contracts.

Additional research is in progress under P. L. 480 funds at The Institute of Plant Protection, Poznan, Poland, (1.5 man-years) and at The Institute Annamalai University, Madras, India (3.0 man-years).

PROGRAM OF THE STATE EXPERIMENT STATIONS

A total of 14.0 professional man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

A. Viral Diseases of Insects

1. Histopathological Studies. Since the discovery of a new nuclear polyhedrosis virus in the cabbage looper larval gut, intensive histopathology has been carried out on Trichoplusia ni and Heliothis zea. The new gut virus, as previously described, forms bundles of rods within a large polyhedron compared to the other virus which has single rods embedded in a much smaller polyhedron. The separation of these viruses is under way and is nearing

completion. It was found that the gut virus polyhedron dissolves more rapidly in relatively weak carbonate solutions than does the single rod embedded virus. Thus through a process of differential dissolution and centrifugation the viruses have been separated.

Polyhedra are rarely found in the gut nuclei of Heliothis zea thus this condition has not been committed to study. However, the nuclear polyhedrosis virus found infecting the hemocoelic tissues have been detected in fat, hypodermal and blood cells as well as in cells of the tracheal matrix.

A new nuclear polyhedrosis virus has been found in the zebra caterpillar Ceramica picta (Harr.) larvae sent from Yakima, Wash. The virosis has been studied in the insect. This virus (embedded as packets of virus rods in the polyhedron) infects the fat body cells, the hypodermis and tracheal matrix. No infected blood cells have been seen to date in the sample examined. No virus has been detected in the gut nuclei. The samples of virus infected larvae were contaminated with a cytoplasmic polyhedrosis that may account for the cross-infection of other species informally reported by the Yakima Fruit and Vegetable Insects Laboratory.

Similar histopathological examinations have been or are being made of salt-marsh caterpillar larvae (Estigmene acrea) infected with granuloses, Fall army worm (Spodoptera frugiperda) and beet armyworm (Spodoptera exigua) infected with their specific nuclear polyhedrosis.

2. Silicon content of nuclear and cytoplasmic viral inclusion bodies causing polyhedrosis in Lepidoptera. Due to the unusual similarity in chemical behavior between the parasporal crystals produced by strains of Bacillus thuringiensis and the viral inclusion bodies causing polyhedrosis in insects, an investigation into the possibility of silicon being present in these bodies was undertaken. The finding of this element in the corn earworm, Heliothis zea (-.12 percent) confirmed the suspicions. An analyses of a number of nuclear and cytoplasmic viral inclusion bodies for this element revealed the following: Both nuclear and cytoplasmic polyhedra isolated from infected Bombyx mori had a silicon content of 0.30 - 0.31 percent; cytoplasmic polyhedra isolated from infected Pectinophora gossypiella had a silicon content of 0.17 percent; nuclear polyhedra taken from Trichoplusia ni contained 0.10 percent silicon.

A study into the chemical behavior under near in vivo conditions revealed that cleaned polyhedra isolated from Bombyx mori, Heliothis zea, and Trichoplusia ni were all dissolved initially by the alkaline components of the insect gut juice, followed by attack of the proteolytic enzymes. Virus rods were released, in all cases, when the polyhedra were incubated with an enzymes inactive alkaline extract of the gut juice. Thus, it was concluded that enzymes contributed little in effecting release of the virus rods except perhaps to hasten the process.

3. Silicon content of the intact granulosis virus from the salt-marsh caterpillar (Estigmene acrea). An analysis of the granulosis virus

infecting the salt-marsh caterpillar revealed a silicon content (Si) of 0.060 percent. This finding was the lowest amount yet described in viral inclusion bodies. A possible correlation between the quantity of silicon present and the time and quantity of alkali required for dissolution and release of the virus particles from their shell was proposed. That is, in vitro, the protein matrix of the viral inclusion bodies is dissolved by treating them with sodium or potassium carbonate. For Bombyx mori polyhedra (0.30 - 0.31 persent Si) the time required for liberation of the virus particles, using a specified concentration of sodium carbonate is 30-60 minutes. Polyhedra isolated from Trichoplusia ni (0.10 percent Si) of Heliothis zea (0.12 percent Si) requires from 1-2 hours. On the other hand, the granulosis virus of the salt-marsh caterpillar requires at least 3 hours of treatment. Thus, it becomes obvious that with increased ocncentrations of silicon, the ease of dissolution by the alkaline constituents of the gut is enhanced. As proposed, the process is probably through conversion of the silicon moiety to a soluble salt of silicic acid. Dissociation of the protein matrix consequently follows.

4. Content of nucleic acid in intact nuclear polyhedral bodies isolated from virus-infected beet armyworms, (Spodoptera exigua). Nucleic acid analyses of intact nuclear polyhedral bodies isolated from infected beet armyworms have revealed the presence of both deoxyribonucleic acid (DNA). and ribonucleic acid (RNA). Total amounts of DNA and RNA were 23.74 $\pm$ 0.11 and 12.69 $\pm$ 0.08 µg/mg of polyhedra, respectively. These findings are in reasonable agreement with other reports of nucleic acid present in polyhedra isolated from other insect species.

5. Human feeding tests. A preparation containing live virus from cotton bollworm larvae, (Heliothis zea), killed by the specific nuclear polyhedrosis was fed to 10 men and women who volunteered as test subjects. Six other men and women (controls) were fed insects protein separated from the same preparation of polyhedral virus. Over the course of five days, each test subject consumed 5.82 billion polyhedra. Complete physical and clinical tests of all subjects and controls were made before the test began and 10 and 30 days after the feeding showed no significant changes in the condition of any individual in the test or control groups and no differences between the subjects and controls were detected.

6. Disease diagnosis - Viruses. The following viruses have been isolated from insects shipped to Beltsville for disease diagnosis:

A cytoplasmic polyhedrosis was isolated from the tobacco budworm (Heliothis virescens) sent from Brownsville, Texas. This may be a new virus.

A nuclear polyhedrosis was obtained from larvae of Malacosoma neustria sent by J. Lipa of Poland.

A nuclear polyhedrosis virus of Faris sp. was isolated from infected larvae sent from Uganda, Africa. This is a new virus isolation and might prove useful if the insect should be accidentally introduced into North America.

Diseased almond moth larvae sent from the Southern Corn Insects Investigations Laboratory were found to contain a new nuclear polyhedral virus which has been isolated and is currently under investigation. A new granulosis virus was isolated from Indian meal moth larvae from the same source. These viruses are being studied intensively here.

B Bacterial Pathogens of Insects

1. Taxonomy of crystalliferous bacteria. Four years continual study of crystalliferous bacteria has culminated in a new key for these bacteria which incorporates the latest findings from most investigators around the world. The salient points are the reduction of Bacillus entomocidus to varietal status based on the discovery of a strain capable of producing acetymethyl-carbinol. The two varieties involved now are classified as Bacillus thuringiensis var. entomocidus Heimpel and Bacillus thuringiensis var. subtoxicus Heimpel. In addition it has become necessary to name four new varieties based on their respective abilities to produce toxins. These are, Bacillus thuringiensis var. amuscatoxicus Heimpel, Bacillus thuringiensis var. azawai Heimpel, Bacillus thuringiensis var. pacificus Heimpel, and Bacillus thuringiensis var. anagastae Heimpel.

The main point of the creation of these subspecies lies in the need of the insect pathologist to be able to distinguish groups of crystalliferous bacteria with different abilities to kill insects. The proposed key attempts to make this distinction, drawing away from the current trend to distinguish these bacteria as academic oddities and in the absence of any critical insect tests.

2. Effect of U.V. Irridiation on Bacillus sotto crystals. Previously, it was found that 10-minute exposure of Bacillus sp. spores to a germicidal U.V. lamp (2537 A) inactivated 99.9% of the spores and that 50% of the spores were inactivated when exposed in sunlight outdoors for 30 minutes. However, tests with the parasporal body (crystal) of B. sotto exposed to U.V. (2537 A) for periods up to 120 minutes did not alter the toxicity of the crystal. Silkworm is used as the bioassay organism because of the extreme toxicity of B. sotto to it and because the silkworm responds only to the crystal.

3. Silicon content of the parasporal crystal of several crystalliferous bacteria. Several proposals have been offered in an attempt to explain the high chemical stability and inertness exhibited by the toxic parasporal crystals of the crystalliferous bacteria. One such proposal is that disulfide bonds, formed during the synthesis and maturation of the crystalline structure, hold it in such a state that the crystal body becomes highly immune to normal chemical degradation. While such a hypothesis seems reasonable as an explanation for the digestion of wool, containing 9-12% cystine, it seems unlikely that the amount of cystine in the crystals (1.1%) could contribute the number of disulfide linkages necessary to form

such highly insoluble, inert structures as the parasporal crystals. In support of this objection is in the fact that soluble proteins such as the albumens contain more cystine (1.5%) than do the parasporal crystals.

With the finding of silicon as a component of these toxic bodies, a more feasible explanation became available. Analyses, both colorimetric and instrumental (Littrow atomic spectrograph) of the crystals produced by several varieties of Bacillus thuringiensis revealed the presence of this element in substantial amounts (0.3-0.4%). An investigation into the solubility properties of the crystals using selective alkaline agents revealed that those compounds commonly used to effect solubilization of the silicates, also were quite effective in dissociating and dissolving the crystals. Alkaline solutions that were not capable of dissolving the silicates, were also incapable of effecting dissolution of the crystals. Thus, it was proposed that in the insect gut, initial dissolution was probably through attack on the silicon moiety by certain alkaline components. Release of the toxic principle then would occur. Unpublished data revealed that gut enzymes alone were incapable of initiating solubilization. The possibility of a secondary action of the proteinases was only speculative.

Dissolution of the toxic parasporal crystals from Bacillus thuringiensis var. variablis by action of the gut secretions of the silkworm, Bombyx mori, on the insect endotoxin produced by Bacillus thuringiensis was undertaken. Using selective experimental conditions, the investigation revealed that dissolution of the proteinaceous crystalline toxin is initially accomplished in the insect gut only through action of the non-enzymatic alkaline components (e.g., potassium and sodium carbonates). Then, the proteolytic enzymes present were capable of attacking and further degrading the crystalline structure. With only the endogenous alkaline components available, 21.42 percent of the crystalline structure was liberated as soluble protein. With a combination of the gut enzymes and alkaline juice, 50.00 percent of the toxic structure was solubilized. No solubilization occurred when the crystals were incubated with the enzymes alone at a pH well within the range of the gut juice.

4. A rapid and sensitive bioassay for Bacillus thuringiensis exotoxin. Last instar house fly larvae, which are 12 to 24 hours away from pupation, are held in plastic petri dishes for determination of infection. Results for high levels of toxicity can readily be observed at the time of pupation. Fleshy or spear pointed anterior portions of the pupae indicate ultimate mortality. Lower toxicity levels not detectable in the pupal stage usually produce adults with vestigal wings and other abnormal morphological characters. CSMA non-asceptically reared flies were equally susceptible to the exotoxin as were flies reared aseptically on a semi-defined diet.

Housefly larvae infected with sublethal doses of exotoxin did not show the characteristic partial flesh-like or anteriorly pointed pupae but emerging adults did have the characteristic vestigal wings. Larvae that were prevented from ingesting exotoxin by a ligature behind the mouth parts

likewise did not exhibit the characteristic partial larval pupae. The route of toxicity in the house fly appears to be by way of ingestion.

Male and female adult house flies were allowed to emerge in a cage having ad libitum dry powdered milk and sugar diet and water containing exotoxin. At 5.0 mg/ml concentration of exotoxin, which is a 100 fold lethal concentration for the larvae, resulted in a continuous mortality over a two-week period. From the fourth day on, the mortality of males was five to ten fold greater than females. These preliminary studies suggest a differential toxicity among the sexes, with the male having the highest mortality and susceptability. Further study in this area is currently under investigation.

The exotoxin was examined as a systemic on kale cuttings against the cabbage looper. Two dosages were used: 5 mg and 10 mg per ml concentration. The larvae on the systemic treated kale fed more voraciously than the controls. The insects were allowed to feed and pupate on the plants and no noticeable differences were observed on emergence of adults either in the systemic treated or controls.

In another experiment, kale was treated with topical application of the lower concentration. After 24 hours equilibration of the cuttings, 5-day-old cabbage loopers larvae were transferred to the leaves. Feeding commenced within a few hours. Mortality was observed in the topically treated leaves within 48 hours and feeding was curtailed.

5. Disease diagnosis - Bacteria. Eleven accessions received during the year contained insects harboring bacteria suspected of being pathogenic. Insects in three of these accessions were found infected with bacteria belonging to the Bacillus cereus group. Two were crystalliferous forms found in both almond moth and Indian-meal moth from accession DD 855 again in Indian-meal moth from accession DD 839 submitted by the Savannah, Ga. Stored Products Insects Laboratory, Market Quality Research Division. Another spore-forming bacterium, apparently a member of the same group, was found in an accession of Earias sp. in which the polyhedrosis virus, previously mentioned, was found. Isolates of these organisms were obtained and will be sent to be sero-typed.

One Enterobacteriaceae infection was found. Tobacco hornworm being reared at the Insect Pathology Laboratory were found to be infected with the red-pigmented bacterium, Serratia marcesens Bizio.

Other bacterial infections found involved nondescript types of bacteria which do not correspond to published descriptions of known species. In most instances these organisms were isolated and when time permits they will be tested for pathogenicity against the hosts, whenever possible, in which they were found. Several isolates of this type of organism were obtained from dead alfalfa weevil larvae. Isolates of similar nondescript types of bacteria were obtained from the white-fringed beetle and the sugarcane borer.

C Fungus Diseases of Insects

1. Disease diagnosis - Fungi. Insects in 9 of the accessions received for the year were found infected with fungi of one type or another. In two cases, we were able to make identification as to species, in three as to genus, and in two as a family. In the other two cases, we were able only to report that an unidentified fungus was present. The fungi which we were able to identify down to genus or species are as follows: Entomophthora sp. on pea aphid collected from vetch; Empusa sp. on unidentified flies collected from dogwood; Penicillium sp., no doubt a saprophyte, on cereal leaf beetle adults from rearing cultures; Metarrhizium anisopliae on predaceous carabid adults from rearing cultures; and Beauveria bassiana on field-collected forest tent caterpillars, on predaceous carabids from rearing cultures, and on field-collected southwestern corn borer larvae. In addition, a single southwestern corn borer larva submitted was found to be infected with a species of Hirsutella. Some of the fungi were cultured and tested against wax moth larvae to facilitate identification. Cultures and permanent slides have been prepared of some of these fungi and added to a collection which will be maintained for reference and study.

2. Undetermined infections. From two accessions received, the insects examined either revealed no organisms or isolation and examination was prevented because of the method used to prepare the specimens for shipment.

No evidence of disease was detected in a shipment of webbing clothes moth larvae received from the Stored-Product Insects Laboratory at Savannah, Ga. on March 3. Many of the larvae had escaped from the container in which they were shipped and only a few larvae were available for examination.

Two red-pigmented Tsetse flies, Glossina morsitans orientalis, suspected of being diseased were received from the Insects of Man and Animals Laboratory in Salisbury, Rhodesia. Both specimens were preserved in alcohol which prevented isolation of possible contributing organisms. Additional specimens were requested.

Since only one nematode infection was found, a separate section for the discussion of nematodes was not warranted. A single specimen of a fall armyworm was submitted by the University of Maryland on September 30. The specimen was identified by the Namatology Laboratory of Crops Research Division as a member of the family Mermithidae.

D Protozoan Infections of Insects

Nosema inactivation by heat treatment. Laboratory tests indicate that spores of Nosema apis can be inactivated by exposure to heat at 120° for six hours. Spores were exposed either in a sucrose solution or dried on a glass slide. Field tests have shown that colonies of bees can be severely infected from combs containing viable Nosema spores. Field tests are now underway to determine if Nosema can be eradicated from equipment used in

apiaries by simply exposing the equipment to sufficient heat to kill the organism without damaging the wax comb.

E. Invitro Cultivation of Insect Tissues

1. Establishment of Lines. There are only 2 lines (cells capable of indefinite in vitro propagation) of insect cells available to investigators at the present time. One was derived from the ovarian tissue of an Asian moth, Antheraea eucalypti, and the other from mosquito tissue. Both of these lines are currently maintained in this laboratory.

Originally both lines required medium supplemented with hemolymph from Antheraea pernyi which occurs only in Asia. Recently sublines of the moth cells, partially or wholly adapted to more readily obtainable supplements have been received through the courtesy of Dr. C. Yunker of the U. S. Public Health Service Laboratory in Hamilton, Mont. One of these sublines grows very well for several weeks in the Grace insect tissue culture medium supplemented with fetal bovine serum before requiring additional supplementation with A. pernyi hemolymph. Methods for the maintenance and growth of this subline have been developed and standardized so that large volumes of these cultures are routinely available for experimental work.

This material is now being used in studies of the nutrition and growth characteristics of this subline. The comparison of these results with those of similar studies to be made with the mosquito line may provide general information applicable to the initiation and maintenance of cell lines from other insect species.

Studies of the primary culture of Lepidopteran tissue. Numerous primary cultures have been initiated using larval and pupal ovaries. These are generally considered to be one of the more useful sources of material for insect tissue culture. Grace's insect tissue culture medium supplemented with 5% Bombyx mori hemolymph was used to culture tissue from Nymphalis antiopa, Estigmene acrea, Laphygma frugiperda, Malacosoma americanum, Galleria mellonella, as well as Bombyx mori.

The availability of these cultures from several species produced under comparable conditions will provide a chance to study the problem of the origin of those cells which migrate and/or multiply to form the useful portion of primary cultures and the material from which cell lines most probably develop. It also will provide material for the study of the developmental stage of the insect most likely to produce successful primary cultures.

F. General

1. Insect collecting kit developed. Under a grant from the World Health Organization, a pocket-sized collecting kit was designed for use in a world-wide survey for protozoan and other agents causing disease among

invertebrates of medical interest. Prototypes were field tested in Australia, Japan, Southeast Asia, United States, and Ghana and one was approved by the World Health Organization for production and distribution to various collectors over the world.

These kits containing diseased material will then be forwarded to the World Health Organization's International Reference Center, Department of Zoology and Entomology, Ohio State University, Columbus, Ohio.

2. Effects of cholesterol deficient diet on the development of the house fly embryo. Embryos from flies fed cholesterol deficient diet failed to develop after the 4th hour, completing less than 1/3 of the normal development period. Most of these embryos died in the early gastrula stage and none live past the 4th hour.

Eggs from females fed a diet with the addition of 0.1% stigmastanol also failed to hatch. However, these embryos were affected differently than those from the cholesterol deficient parents. They appear to develop normally up to 3 hours; then development lags behind that of normal embryos, the most striking effect being in the mid-gut. At this time, instead of becoming a closed, highly coiled tube, the mid-gut remains an open straight tube.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Diseases of Insects

Cantwell, George E., David A. Knox, T. Lehnert, and A. S. Michael. 1966. Mortality of honey bee (Apis mellifera L. Hymenoptera: Apidae) colonies treated with some biological insecticides. J. Invert. Pathol. 8: 228-33.

Cantwell, George E. and Marshall Laird. 1966. The World Health Organization Kit for the collection and shipment of pathogens and parasites of diseased vectors. World Health Organ./EBL/66.61.

Estes, Z. E., and R. M. Faust. 1966. Silicon content of intact nuclear polyhedra from the corn earworm, Heliothis zea. J. Invert. Pathol. 8: 145-49.

Faust, R. M., and Z. E. Estes. 1966. Nucleic acid composition of the nuclear-polyhedrosis virus from Trichoplusia ni. J. Invert. Pathol. 7: 521-22.

Faust, R. M. 1966. Content of nucleic acid in intact nuclear polyhedral bodies isolated from virus-infected beet armyworms, Spodoptera exigua (Lepidoptera: Noctuidae). J. Invert. Pathol. 8: 273-74.

Heimpel, A. M. 1966. Exposure of white mice and guinea pigs to the nuclear polyhedrosis of Trichoplusia ni (Hubner). J. Invert. Pathol. 8: 98-102.

Ignoffo, Carlo M., and Jean R. Adams. 1966. A cytoplasmic-polyhedrosis virus Smithiavirus pectinophorae sp. n of the pink bollworm, Pectinophora gossypiella (Saunders). J. Invert. Pathol. 8: 59-66.

General

Cantwell, George E., T. J. Shortino, and W. E. Robbins. 1966. The hostopathological effects of certain carcinogenic 2-fluorenamine derivatives on house-fly larvae. J. Invert. Pathol. 8: 167-74.

Cantwell, George E., and B. A. Franklin. 1966. Inactivation by irradiation of spores of Bacillus thuringiensis var. thuringiensis. J. Invert. Pathol. 8: 256-58.

Connor, Ray M. and P. Arne Hansen. 1966. Effects of Valine, Leucine, and Isoleucine on fly toxin production by Bacillus thuringiensis Berliner and related varieties. Ph.D. Thesis, University of Maryland. 66 p.

Estes, Z. E., and R. M. Faust. 1965. A colorimetric method for the determination of silicon in biological materials. Anal. Biochem. 13: 518-22.

Faust, R. M., and Z. E. Estes. 1966. Silicon content of the parasporal crystal of several crystalliferous bacteria. J. Invert. Pathol. 8: 141-44.

Heimpel, A. M. 1966. Microbial control of insects. World Rev. Pest Control. 4: 150-61.

PUBLICATIONS -- STATE EXPERIMENT STATIONS AND COOPERATIVE PROGRAMS

Flanders, S. E. and I. M. Hall. 1965. Manipulated bacterial epizootics in Anagasta populations. J. Invert. Pathol. 7: 368-77. (Calif.)

Harvey, T. L. and D. E. Howell. 1965. Resistance of the house fly to Bacillus thuringiensis Berliner. J. Invert. Pathol. 7: 92-100. (Okla.)

Kulman, H. M. and Marion A. Brooks. 1965. Dosages of nuclear-polyhedrosis virus effective against Malacosoma disstra with notes on interspecies susceptibility. J. Econ. Entomol. 58: 1008-1010. (Va.)

Pitts, C. W. and T. L. Hopkins. 1965. Lipid composition and hibernation of face flies, Musca autumnalis. Bull. Entomol. Soc. Amer. 11: 159.(Kans.)

AREA NO. 20. INSECT PHYSIOLOGY AND MODE OF ACTION OF INSECTICIDES AND THEIR METABOLITES

Problem. Basic research in insect physiology is essential to the development of more efficient insecticides and new approaches to insect control. The increasing development of resistance to insecticides by insects has emphasized the need for additional information on the mode of action and metabolism of insecticides in insects and the mechanisms of the resistance to insecticides. More knowledge is also needed on the normal physiology and biochemistry of insects to permit a comparison and interpretation of the data obtained from studies on insect toxicology. Basic research in insect biochemistry and physiology, including insect nutrition and metabolism, will provide a better understanding of the biochemical and physiological systems which regulate insect growth, metamorphosis, reproduction and diapause, and the chemistry and action of the hormones which mediate these systems. Knowledge gained from such research is essential to the development of new methods of effective insect control which are safer and more selective in their action than the methods now being used. More basic information on the response of insects to light, sound, food, and sex attractants could contribute to better insect control. Insects are useful test animals for basic physiological studies on life processes.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving insect physiologists, biologists, geneticists and chemists engaged in basic studies in insect physiology and biochemistry and in the mode of action of insecticides and their metabolites. At the Pioneering Research Laboratory on Insect Physiology at Beltsville, Md., basic research is conducted on biochemistry and physiology of lipids in insects, insect hormones, and nutrition, at the Metabolism and Radiation Research Laboratory at Fargo, N. D., on metabolism of insecticides and other compounds in insects and physiological studies on insects and in the Pesticide Chemicals Research Branch at Beltsville on insect biorhythms.

The Federal scientific effort devoted to research in this area totals 16.6 scientist man-years. Of this number 3.2 is devoted to the biochemistry and physiology of lipids in insects, 3.3 to insect hormones, 0.5 to nutrition, 3.0 to metabolism of insecticides, 2.0 to physiological processes specific to insects, 1.8 to physiology of insect growth and development and 2.8 to insect biorhythms.

Additional research in this area is provided by the following P. L. 480 projects: S5-ENT-3 Colombia (2 professional man-years); A7-ENT-6 India (2 professional man-years); A7-ENT-14 India (2 professional man-years); E21-ENT-3 Poland (1 professional man-year); and E21-ENT-4 Poland (1 professional man-year); and a research contract with the Mississippi State University, No. 12-14-100-6895(33) (0.3 man-year).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 48.0 professional man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

Insect Physiology Pioneering Research Laboratory

A. Biochemistry and Physiology of Lipids in Insects

1. The Isolation and Identification of the Sterols of the DD-136 Insect Parasitic Nematode and Their Derivation From Its Insect Hosts. A broad program was initiated at Beltsville, Md., in cooperation with the Pioneering Research Laboratory on Insect Pathogens to study the biochemical interaction between certain pathogens and parasites and their insect hosts. Sterols of the nematode DD-136 and a host, the greater wax moth (Galleria mellonella (Linnaeus)), were investigated. The sterol fraction of the wax moth larvae when analyzed by gas-liquid chromatography (GLC) showed the presence of one major sterol, with the relative retention time (RRT) of cholesterol and trace amounts of campesterol and β-sitosterol. Analyses of the sterol fraction from the nematode showed the presence of two sterols tentatively identified by their RRT's as cholesterol and Δ^7-cholestenol. The sterol content of the infective stage larvae was found to be about 95 picograms (9.5×10^{-11} grams) per nematode as determined by GLC analysis and radiotracer techniques.

The sterols were isolated from 69.6 grams of nematodes (3.3×10^8 organisms) and the cholesterol and Δ^7-cholestenol were separated by column chromatography and crystallized. The two crystalline sterols were positively identified, as the free sterols and acetates, by melting points, mixed melting points, optical rotation, infrared spectra, and GLC analyses by three systems.

To determine whether the sterols of the nematode were derived from those of the host, wax moth larvae were injected with 4-C^{14}-labeled cholesterol before exposure to the associated bacterium and to the infective larvae of the nematode. The nematodes were harvested, the sterols were isolated as above and analyzed by GLC with concurrent trapping of the radioactive compound. Both the cholesterol and the Δ^7-cholestenol samples were found to be radioactive, with approximately the same specific activity. The microorganism associated with the nematode when tested alone did not effect a change in either the total sterol content or the types of sterol present in the insect but did bring about an appreciable increase in esterified sterols. These results indicate that (1) the nematode does derive its sterol from the insect host and (2) does convert a part of the cholesterol from the host to Δ^7-cholestenol.

2. The Demonstration of Sterols as Requirements for the Growth, Development, and Reproduction of the DD-136 Nematode. The above study indicated that the DD-136 nematode derives its sterol from the insect host and converts a part

of the cholesterol from the host to Δ^7-cholestenol. Since these tests did not establish whether or not an exogenous source of sterol is essential for normal growth and development, nutritional studies were undertaken to determine this. Although a chemically defined medium adequate for growth and development has not yet been attained, a test system was devised in which living or dead cells of the associated bacterium served as a source of nutrient for the nematode. The two media used to support the nematode and to culture the bacterial cells, and the bacterial cells as well, were found to contain only trace amounts of sterol. Thus in this system it was possible to study the sterol requirement of the nematode.

Nematodes added to slants containing the associated bacterium, both with and without the addition of cholesterol, exsheathed and initiated development a few hours after introduction and reached the adult stage in about four days. The nematodes without added cholesterol did not increase further in size, and although the females produced ova, the ova failed to hatch. However, those nematodes on the medium to which cholesterol had been added continued to develop to approximately a four-fold increase in size and produced viable eggs. The eggs from these adults hatched, and the progeny produced several additional generations of normal nematodes without further transfer.

Ten highly purified sterols were tested by this method for support of nematode growth and reproduction, and all the compounds tested could replace cholesterol in supporting growth and reproduction except stigmasterol and ergosterol. These two compounds are similar in structure and differ from the other sterols tested in having a double bond at the 22,23-position. Two sterols that contain a double bond at the 7,8-position were slightly superior to cholesterol.

The results of these studies definitely established that: (1) The DD-136 nematode requires an exogenous source of sterol for normal growth and reproduction and (2) although a number of sterols do fulfill this requirement, structural specificity exists. This information will be useful in the development of a more satisfactory artificial medium for mass rearing nematodes for insect control.

3. Relationship Between the Sterols of the Virginia Pine Sawfly to Those of Two Host Plants. Studies of this relationship were cooperative with the Forest Insects Laboratory, FS at Beltsville. Analysis of the sterols of larvae of the Virginia pine sawfly indicated cholesterol (73%) to be the major sterol, with β-sitosterol (17%), 7-dehydrocholesterol (6%), and campesterol (4%) also present. The sterol content of the foliage of two host plants, the Virginia pine and the pitch pine, were also examined and found to contain primarily β-sitosterol and campesterol, with β-sitosterol accounting for greater than 90% of the total sterol in both plants. Trace amounts of cholesterol (<1%) were found in Virginia pine foliage but no cholesterol was detected in the foliage of the other host. No biosynthesis of sterols was detected in the larvae when C^{14}-labeled acetate, mevalonate,

or glucose were tested as precursors. This indicates that the sawfly, like all other insect species examined to date, must rely on an exogenous or dietary source of sterol for normal growth and development. The larvae were fed H^3-labeled β-sitosterol and were found to convert this sterol to H^3-labeled cholesterol and 7-dehydrocholesterol. The sawfly then dealkylates β-sitosterol obtained from the host plant to cholesterol and obtains its required cholesterol in this manner.

B. Insect Hormones

1. Juvenile and Gonadotropic Activity of Epoxy-Farnesol Derivatives. A number of isoprenoid derivatives which exhibit extremely high juvenile and gonadotropic hormone activities have been synthesized and tested at Beltsville. Introduction of an epoxide moiety at the 10,11 position of farnesol, farnesyl methyl ether and the methyl ester of farnesenic acid was found to greatly enhance the juvenile hormone mimicking activities of these compounds. The 10,11 epoxide of trans,trans-farnesenic acid methyl ester was the most active compound discovered. It is 1,600 times as active as farnesol and in certain insects is the most active synthetic compound of known structure reported to date. As little as 30 nanograms of the epoxy ester applied topically to the yellow mealworm inhibits normal development of the genitalia and many of the test organisms die shortly after molting to the adult beetle. In most of the surviving beetles the genital modification is serious enough to prevent normal copulation and reproduction. The 6,7 epoxide of farnesenic acid methyl ester was inactive in the Tenebrio test, indicating the importance of the 10,11 position.

The 10,11 epoxide of farnesenic acid methyl ester was also found to have extremely high gonadotropic (ovarian maturation) hormone activity in allatectomized American cockroaches. The compound reinitiates ovarian development and yolk formation at levels as low as 0.1 - 0.2 microgram. The consistently strong gonadotropic effect found for this compound has previously been obtained only with purified preparations of the Cecropia hormone.

It was discovered that all of the epoxides were more active by topical application than by injection. The fact that these compounds appear to readily penetrate the insect cuticle and interfere with the development of a number of insects at extremely low levels provides a basis for their potential economic use in insect control either directly as chemical control agents or as a guide in the development of more potent insect hormone mimics.

2. A Modified Assay for Molting Hormone Activity. A modified molting hormone assay patterened after the Calliphora test was developed at Beltsville in which the larva of the house fly was used as the test insect. The house fly was found to have a number of advantages over Calliphora: The house fly is more easily reared and maintained and requires less space. A single container of house fly larvae (approximately 1750) reared by the

CSMA procedure usually afforded sufficient insects for ligation and testing. Four to eight times as many organisms suitable for use in assay were obtained with the house fly than with Calliphora. In a series of 111 tests over a two-year period, in which approximately 56,000 larvae were ligated by twelve different operators, the overall average yield of test insects was 20%. With experienced operators, however, yields of 35-40% were not unusual. Furthermore, the shorter time interval required for the detection of spontaneous development in ligated house fly larvae permitted injections to be made on the day following ligation, thus reducing both the time required for assay and mortality that could result from a longer holding period.

In addition to the ease in rearing and handling and the high yields of ligated insects suitable for bioassay, the house fly assay was found to be three to four times more sensitive. A house fly unit (that amount of crystalline α-ecdysone resulting in 60% pupation of the test organisms) was found to be 0.005-0.006 μg as opposed to 0.02 μg for the Calliphora unit. The successful use of the house fly assay both for titer determination in a number of species and in the isolation of crystalline ecdysone from the tobacco hornworm definitely establishes the usefulness of this assay as a tool for research in insect endocrinology.

3. Ecdysone Titer in the Pupa of the Tobacco Hornworm. The titer of ecdysone(s) is known to vary quantitatively in the different stages of insects and in the case of holometabolous forms the hormone titer has been shown to be high during certain periods of pupal development. Ecdysone titer was determined at Beltsville in tobacco hornworm pupae of different ages, beginning at zero time (when the prepupal skin was shed) and then on alternate days through day 20, or one day prior to emergence. The ecdysone was extracted from groups of pupae and the extracts partially purified. Bioassay of the purified extracts using the house fly assay showed the presence of a single sharp peak in titer at 6-8 days after pupation. Countercurrent distribution and bioassay of the extracts at maximum titer revealed the presence of two biologically active components.

The average maximum titer in hornworm pupae was found to be approximately 425 house fly units per gram of tissue. This calculates to be about 2 μg of ecdysone per gram of tissue, or nearly ten times any single maximum hormone titer found for the silkworm pupa. The hornworm also differs from the silkworm in that the latter insect has two distinct peaks in hormone titer during the pupal stage. It is currently not known whether or not the multiple peaks in titer reported for the silkworm and other insects represent different or distinct ecdysones. It is possible, however, that the presence of two different ecdysones in the single observed hormone maximum in the hornworm pupa may be analogous to the presence of more than one maxima found for other insects.

4. Isolation and Identification of the Molting Hormones From the Tobacco Hornworm. Two crystalline ecdysones were isolated from 12.7 kg (wet weight)

of 7-day-old hornworm pupae at Beltsville. The two hormones were found to have equal biological activity in both the Calliphora test and the house fly assay. The physical properties, including m.p., K values, and UV, IR, NMR and mass spectra, were taken on both compounds. One of the hormones was found to be identical in both physical properties and biological activity to the silkworm α-ecdysone (2β, 3β, 14α, 22R, 25-pentahydroxy, Δ^7-5β-cholesten-6-one). The more polar hormone, present in greater amounts (55-80%) in 7-day-old hornworm pupae, was structurally similar to α-ecdysone but based on spectral data was assigned an additional hydroxyl group at the C-20 position. Although partially purified fractions with molting hormone activity have been prepared from several insects, prior to this work only the ecdysones from the silkworm pupa and the adult Moroccan locust have been isolated in crystalline form.

The more polar ecdysone from the hornworm was found to have certain physical properties similar to the polar ecdysones isolated from insects and from a crustacean. Differences exist, however, in both the physical properties and biological activities of these compounds which cannot currently be reconciled from the data at hand. A final decision on the relationship among these ecdysones must await additional information about their structure. Work is currently underway to confirm the structure of the polar ecdysone from the tobacco hornworm.

The significance of the presence of more than a single molecular species with molting hormone activity in an insect is not presently understood. The different ecdysones could represent intermediates in the biosynthetic-degradative scheme. Alternatively, each of these compounds could be an active hormone with a specific physiological function. A more complete knowledge of the structural and functional diversity of the insect ecdysones is essential to a better understanding of their roles in insect development and their eventual exploitation for insect control.

C. General

1. Effect of Triparanol on Sterol Metabolism and Atherosclerosis in the Mongolian Gerbil. Mongolian gerbils injected with Triparanol, an inhibitor of cholesterol biosynthesis, showed a decrease in blood and liver cholesterol when compared to controls. As the dosage of Triparanol was increased, the concentration of desmosterol ($\Delta^{5,24}$-cholestadiene-3β-ol) increased in both serum and liver. Altherosclerotic lesions of the aorta were graded microscopically. There were no lesions found in the control animals, while the number of lesions increased with increased dosages of Triparanol. These results show that Triparanol, which was previously proposed and used as an antiatherosclerotic agent, actually increases the incidence of atherosclerotic lesions in this experimental animal. This study was conducted in cooperation with the Department of Physiology, Howard University, College of Medicine.

Metabolism and Radiation Research Laboratory

D. Metabolism of Insecticides and Other Compounds in Insects

1. Insect Chemosterilants. A series of studies on the effects of chemosterilants on the physiology of insects was initiated. An ultramicro colorimetric method was developed for the determination of aziridine chemosterilants. Reproducible standard curves were obtained with three chemosterilants at the following range of concentrations: tepa 0.05 to 1.0 µg; metepa 0.0625 to 0.5 µg; and tretamine 0.05 to 1 µg. Aqueous solutions of tepa and metepa were stable for at least 48 hours, while those of tretamine indicated a breakdown of the parent material up to 70% in 24 hours. The advantage of this ultramicro test is that very small quantities in the order of 10 µl or less are required. A major drawback of this technique is that the mono-, di-, and triethylene amino compounds cannot be differentiated as any intact aziridinal group will give the color reaction. However, this test is useful in that the breakdown of the parent compound can be followed.

Amino acid analysis of hemolymph from cabbage looper larvae indicated that autoxidation occurred unless the collection and sample preparation were done under nitrogen. To facilitate hemolymph collection and dissection, an inexpensive nitrogen box was designed which can be used with a dissecting microscope.

E. Physiological Processes Specific to Insects

1. Lipid Mobilization in Insects. An *in vitro* study of lipid mobilization in the cockroach employing gravimetric and radioisotope methods has demonstrated the release of triglycerides from the fat body upon incubation with hemolymph. On the basis of time and hemolymph concentration studies, the mobilization of triglycerides appeared to be hemolymph specific compared to the non-specific diffusion of mono- and diglycerides. Fat body triglycerides showed an optimum mobilization release when incubated for 30 minutes with 50% hemolymph/ml of incubation media.

2. Isolation and Identification of Insect Neurohumoral Substances. Work has continued on a neuroactive substance isolated by the Von Euler extraction procedure for catecholamines from the house fly and the cockroach. Bioassay with the ventral nerve cord and motor nerves of the cockroach gave a marked response with the unknown substance. In several preliminary experiments with a nerve-muscle preparation from the cockroach, the neuroactive material potentiated the mechanical response of the indirectly stimulated muscle. Chemical characterization of this material was difficult because of trace contaminants which appeared in chromatographic separation procedures. The chemical identification of catecholamines in the house fly, cockroach, and cabbage looper is continuing. Noradrenaline and dopamine were found in all three species while 3-4-dihydroxyphenyl

acetic acid and N-acetyl dopamine were present only in the house fly and cockroach. Noradrenaline had an effect on the central nervous system of the cockroach at a concentration of 2×10^{-5} M, suggestive of neurotransmitter function in this insect.

3. Fatty Acid Analysis of Lipids. As part of the background study on lipid metabolism in insects, an analysis was made of the fatty acids of the neutral lipid fractions of the fat body and hemolymph in the 4th and 8th instars as well as young adults of both sexes of the American roach. The fatty acids were extracted with chloroform-methanol and petroleum ether, passed through a Florisil column, transesterified, and finally analyzed by gas chromatography. The major saturated fatty acids in both the fat body and hemolymph fractions were palmitic and stearic, while the major unsaturated fatty acids were palmitoleic, oleic, and linoleic, with oleic predominating.

The triglyceride fraction in both the fat body and hemolymph increased in both sexes from the 4th to the 8th instar and dropped to over half the 4th instar value in the young adult stage. A similar change was seen in the monoglyceride fraction of the fat body and hemolymph. In contrast to the triglycerides, monoglycerides, and free fatty acid fractions, the diglyceride fraction decreased in the 8th instar but again increased in the young adults.

It was evident from these studies that total lipid or triglyceride analysis is not indicative of the changes occurring in all the individual glyceride and free fatty acid fractions. The changes in the diglyceride fractions may reflect anabolic and catabolic changes occurring in the triglycerides.

4. Cuticular Wax Formation in Insects. Some preliminary experiments with radioactive precursors, i.e. potassium palmitate-C^{14} and sodium acetate-C^{14}, were made to study hydrocarbon synthesis and its incorporation in cuticular wax. In vivo experiments with the American roach indicated that acetate was more efficient as a precursor of hydrocarbons than palmitate, while the reverse was true for lipid synthesis. In vitro experiments with labeled acetate indicated that no hydrocarbon synthesis occurred with incubates of whole or homogenized fat body. On the other hand, hydrocarbon synthesis was observed when integument was incubated in vitro with acetate but not observed when homogenates of the integument were used.

F. Physiological Studies on Insect Growth and Development

1. Insect Tissue Culture. An insect tissue culture laboratory was set up and experiments initiated with organ cultures, cell dispersion, and embryonic tissue culture of the cockroach, house fly, and grasshopper. So far, seven different culture media—some holidic and some meridic—have been evaluated. Cultures of embryonic tissue from the cockroach are still

living after over 150 days in vitro. Initial tests with house fly tissue are encouraging with survival after 50 days in vitro. Organ cultures of grasshopper embryo tissue survived after 21 days and showed mitotic activity.

Experiments with the effects of endocrine gland extracts on cockroach leg regenerates are almost completed. Over 250 leg regenerates and 12 combinations of tissue extracts were made. Significant differences in the reaction of the leg regenerates, i.e. stimulation or stoppage of growth, to these combination of extracts were found. The data are now being analyzed.

Attempts to develop subcultures of pure epithelial cells in a monolayer appeared to be successful. Flat sheets of cells were also produced by outgrowth of organ cultures. The mitotic activity of these cells in the monolayer (grown in a Rose chamber) was recorded using low power, low speed time lapse cinephotography. An index of mitosis expressed in terms of mitosis/cell/hour was used, and the range obtained was 1 mitosis/142 cell hours to 1 mitosis/900 cell hours. When this index was plotted against the age of the tissues in vitro, it became apparent that the rate of mitosis decreased with increasing age of the tissues in vitro. It appears likely that the declining rate reflects a continuing differentiation of the cells in vitro rather than the dedifferentiation into embryonic fibroblasts commonly found in vertebrate tissue culture.

2. Bioassay and Extraction of Substance Which Prevents Second Matings in Female House Flies. Since monogamy in female house flies is induced by a secretion produced in the male copulatory duct which is transferred during copulation, extracts were prepared from house fly testes, male copulatory ducts, and virgin female and mated female reproductive systems, exclusive of the ovaries. These extracts were injected into the thorax of 3-day-old virgin females to determine the effects on mortality and mating. The testes extract had an LD_{50} value of 2.5 tissue equivalents and those for the copulatory duct extract 4.5. Only the copulatory duct extract and the mated female extract inhibited mating, with 50% inhibition occurring at 4.25 and 3.75 tissue equivalents respectively. Previous experiments showed that implantation of one copulatory duct in the virgin female produced 50% inhibition of mating. Studies are now in progress to develop a more efficient extraction procedure for isolation and identification of the chemical involved in mating inhibition.

Pesticide Chemicals Research Branch

G. Insect Biorhythms

1. Light Penetration of Fruit, Vegetables, Cotton Squares, and Soil. In cooperation with Market Quality Research Division, basic information was obtained at Beltsville, Md., on percent transmission of light of different

wavelengths in infested and non-infested cotton bolls, apples, peaches, pecans, tomatoes, grapes, cucumbers, and corn stalks. The light transmission was mostly in the red area. This information will be helpful in studies on the manipulation of light to cause or break diapause in the pink bollworm, the corn earworm, the oriental fruit moth, and other insects.

2. The Spectral Characteristics of Light Most Effective in Controlling Diapause in Insects. This is a long-term investigation at Beltsville in cooperation with Market Quality Research Division scientists who built and assembled the apparatus for the study. Three units are now in use for the study of the action spectrum on the codling moth. Further work is planned on the oak silkworm, the corn earworm, the pink bollworm, and the oriental fruit moth.

3. The Circadian Rhythm of Sensitivity of House Flies and Cockroaches to Insecticides. This experiment is being conducted in Beltsville in cooperation with the University of Minnesota. Random samples of conditioned house fly and Madeira cockroach populations were exposed concomitantly to the same dosage of pyrethrins, 40 mg/1000 ft^3, every hour over a 24-hour period. Additional samples were treated in the same manner for the next two days from 8:00 a.m. to 8:00 p.m. Early results indicate that the peak sensitivity to pyrethrins for these insects occurs during the daylight hours.

4. Chemical Changes in Insects in Relation to Biorhythms and Diapause. Some of the differences between diapausing and non-diapausing corn borers and codling moths have been investigated at Beltsville using disc electrophoresis and oxygen uptake as techniques. Preliminary observations suggest that the concentration of proteins in hemolymph of diapausing insects is lower than that in insects at the same stage of development which are about to pupate. Preliminary work also indicates that in the standardized Madeira roach, the concentration of protein in the hemolymph varies during the 12 hour day--12 hour night cycle used for standardization.

Advice in planning and carrying out biorhythm experiments is being given to other laboratories of this division as follows: The effect on harmful insect populations of manipulating photoperiods in bioclimatic cabinets at Brownsville, Texas; the effect on honey bee populations of manipulating photoperiods in honey bee flight rooms at Logan, Utah; and methods for breaking light-induced diapause in the European corn borer at Ankeny, Iowa.

PUBLICATIONS — USDA AND COOPERATIVE PROGRAM

Biochemistry and Physiology of Lipids in Insects

Bowers, W. S. and M. J. Thompson. 1965. Identification of the major constituents of the crystalline powder covering the larval cuticle of Samia cynthia ricini (Jones). J. Insect Physiol. 11: 1003-11.

Schaefer, C. H., J. N. Kaplanis, and W. E. Robbins. 1965. The relationship of the sterols of the Virginia pine sawfly, Neodiprion pratti Dyar, to those of two host plants, Pinus virginiana Mill and Pinus rigida Mill. J. Insect Physiol. 11: 1013-21.

Insect Hormones

Vroman, Hugh E., J. N. Kaplanis, and W. E. Robbins. 1965. Effect of allatectomy on lipid biosynthesis and turnover in the female American cockroach, Periplaneta americana (L.). J. Insect Physiol. 11:897-904.

Bowers, William S., Malcolm J. Thompson, and Edward C. Uebel. 1965. Juvenile and gonadotropic hormone activity of 10,11-epoxyfarnesenic acid methyl ester. Life Sciences 4: 2323-31.

General

Wong, H. Y. C., H. E. Vroman, and H. C. Mendez. 1966. Atherogenic aspects of desmosterol metabolism caused by prolonged triparanol administration. Life Sciences 5: 629-37.

Cantwell, George E., T. J. Shortino, and W. E. Robbins. 1966. The histopathological effects of certain carcinogenic 2-flourenamine derivatives on larvae of the house fly. J. Invert. Pathol. 8: 167-74.

PUBLICATIONS - STATE EXPERIMENT STATIONS
and COOPERATIVE PROGRAMS

Adams, J. R., P. R. Holbert and A. J. Forgash. 1965. Electron microscopy of the contact Chemoreceptors of the stable fly. Stomoxys calcitrans (L.) Ann.Entomol. Soc. Amer. 58: 909-17.(N.J.)

Addy, N. D. and A. G. Richards. 1965. Negative results from treating the American cockroach, Periplaneta americana, with carragenin. Ann. Entomol. Soc. Amer. 58: 402-3. (Minn.)

Anderson, John F. and William R. Horsfall. 1965. Dimorphic development of transplanted juvenile gonads of mosquitoes. Science 147: 624-5. (Conn.)

Barnes, W. W. and G. W. Ware. 1965. The absorption and metabolism of C^{14}-labeled endosulfan in the house fly. J. Econ. Entomol. 58: 286-91. (Ohio)

Beard, R. L. 1965. Ovarian suppression by DDT and resistance in the house fly. (Musca domestica L.). Entomol. Exp. Appl. 8: 193-204. (Conn.)

Blum, M. S. and G. N. Ross. 1965. Chemical releasers of social behavior V. Source, spcificity, and properties of the odour trail pheromone of Tetramorium guincense (F.). (Formicidae Myrmicinae). J. Insect Physiol. 11: 857-68. (La.)

Bowery, T. G., P. E. Catterdam, F. E. Guthrie and R. L. Rabb. 1965. Metabolism of insecticidal residues--fate of inhaled C^{14}-TDE in rabbits. J. Agr. Food Chem. 13: 356-59. (N.C.)

Brooks, Marion A. 1965. The effects of repeated anathesia on the biology of Blatella germanica (Linnaeus). Entomol. Exp. Appl. 8: 39-48. (Minn.)

Bruno, C. F. and D. G. Cochran. 1965. Enzymes from insect tissues which catabolize pyrimidine compounds. Comp. Biochem. and Physiol. 15: 113-24. (Va.)

Button, Grace M., R. Glenn Brown, Frances G. Michels and John T. Smith. 1965. Utilization of calcium and sodium sulfate by the rat. J. Nutrition. 87: 211. (Tenn.)

Campbell, J. E. and D. M. Daugherty. 1965. Observation of spermatophore production in the red grasshopper mite Eutrombidium trigonum (Hermann) (Acarina: Trombidiidae). Ann. Entomol. Soc. of Amer. 58: 824-25. (Mo.)

Chippendale, G. Michael and Stanley D. Beck. 1965. Nutrition of the cabbage looper. I. Some requirements for larval growth and wing development. J. Insect Physiol. 11: 211-23. (Wisc.)

Cook, B. J. and A. J. Forgash. 1965. The identification and distributions of the carboxylic esterases in the American cockroach, Periplaneta americana (L.) J. Insect Physiol. 11: 237-50. (N.J.)

Dadd, R. H. and T. E. Mittler. 1965. Studies on the artificial feeding of the aphid Myzus persicae (Sulzer) - III. Some major nutritional requirements. J. Insect Physiol. 11: 717-43. (Calif.)

Daly, H. V. and A. Sokoloff. 1965. Labiopedia, a sex-linked mutant of Tribolium confusum. J. Morphol. 117(2): 251-70. (Calif.)

Downe, A. E. R. 1965. Notes on the effects of interrupted and multiple feeding on digestion of vertebrate serum proteins in mosquitoes (Diptera: Culicidae). J. Kans. Entomol. Soc. 38(3): 284-86. (Kans.)

Georghiou. G. P. 1965. Development and characterization of resistance to O-isopropoxyphenyl methylcarbamate in the mosquito Culex pipiens quinquefascinatus Say. Nature. 207: 883-4. (Calif.)

Georghiou, G. P. 1965. Genetic studies on insecticide resistance. Advances in Pest Control Res. 6: 171-230. (Calif.)

Good, E. E., G. W. Ware, and D. F. Miller. 1965. Effects of insecticides on reproduction in the laboratory mouse: I Kepone. J. Econ. Entomol. 58: 754-57. (Ohio)

Hagen, K. S. and R. L. Tassan. 1965. A method of providing artificial diets to Chrysopa larvae. J. Econ. Entomol. 58: 990-1000. (Calif.)

Hays, S. B. 1965. Some effects of reserpine, a tranquilizer, on the house fly. J. Econ. Entomol. 58: 782-83. (S.C.)

Hitchcock, Stephen W. 1965. Field and laboratory studies of DDT and aquatic insects. Conn. Agr. Exp. Sta. Bull. 668. 32 pp. (Conn.)

Hodgson, Ernest. 1965. The phospholipids of the tobacco hornworm, Protoparce sexta Johan. (Lepidoptera: Sphingidae). Experimentia 21: 78. (N.C.)

Howell, D. E. and L. D. Goodhue. 1965. A simplified insect olfactometer J. Econ. Entomol. 58: 1027-28. (Okla.)

Ishii, T. and M. Sherman. 1965. Resistance of a Hawaiian strain of the German cockroach to several insecticides. J. Econ. Entomol. 53: 46-50. (Hawaii)

Ishida, M. and P. A. Dahm. 1965. Purification and stereospecificity of house fly enzymes. J. Econ. Entomol. 58: 602-7. (Iowa)

Ishida, M. and P. A. Dahm. 1965. Metabolism of benzene hexachloride isomers and related compounds in vitro. 1. Properties and distribution of the enzyme. J. Econ. Entomol. 58: 383-92. (Iowa)

Judson, C. L., Y. Hokama, and L. Haydock. 1965. The physiology of hatching of mosquito eggs: Some larval responses to the hatching stimulus. J. Insect Physiol. 11: 1169-77. (Calif.)

Kanungo, L. 1965. Oxygen uptake in relation to water balance of a mite (Echinolaelaps echidninus) in unsaturated air. J. Insect Physiol. 11: 557-68. (Ohio)

Knulle, W. 1965. Die Sorption and Transpiration des Wasserdampfes bei der Mehlmilbe (Acarus siro L.). Z. Vergl. Physiol. 49: 586-604. (Ohio)

Lambremont, Edward N. 1965. Biosynthesis of fatty acids in aspetically reared insects. Comp. Biochem. Physiol. 14: 419-24. (La.)

Lichtenstein, E. P. 1965. Problems associated with insecticidal residues in soils. Academic Press: "Research in Pesticides" pp 199-205. (Wisc.)

Lichtenstein, E. P. 1965. Persistence and behavior of insecticidal residues in soils and their translocation into crops. Archiv. of Environm. Health (A.M.A.) 10 825-26. (Wisc.)

Lichtenstein, E. P. and K. R. Schulz. 1965. Insecticide persistence and translocation, residues of aldrin and heptachlor in soils and their translocation into various crops. J. Agr. & Food Chem. 13: 57-63. (Wisc.)

Mehendale, H. M. and Ernest Hodgson. 1965. Metabolism of carnitine in the blowfly, Phormia regina. J. Elisha Mitchell, Sci. Soc. 81: 81. (N.C.)

Metcalf, R. L. and T. R. Fukuto. 1965. Effects of chemical structure on intoxication and detoxication of phenyl N-methylcarbamates in insects. J. Agr. Food Chem. 13: 220-31. (Calif.)

Metcalf, R. L. and M. Frederickson. 1965. Selective insecticidal action of isopropyl parathion and analogues. J. Econ. Entomol. 58: 143-7. (Calif.)

Metcalf, R. L., T. R. Fukuto, M. Frederickson, and L. Peak. 1965. Insecticidal activity of alkylthiophenyl N-methylcarbamates. J. Agr. Food Chem. 12: 473-77. (Calif.)

Michels, Frances G. and John T. Smith. 1965. A comparison of the utilization of organic and inorganic sulfur by the rat. J. Nutrition 87: 217. (Tenn.)

Mills, R. R. and D. G. Cochran. 1965. Isolation and properties of inorganic pyrophosphatases from cockroach thoracic muscle mitochondria. J. Insect Physiol. 11: 1463-74. (Va.)

Mittler, T. E. and R. H. Dadd. 1965. Assimilation by apterous adult Myzus persicae maintained on a synthetic diet. Ann. Entomol. Soc. Amer. 58: 396-401. (Calif.)

Mittler, T. E. and R. H. Dadd. 1965. Differences in the probing responses of Myzus persicae (Sulzer) elicited by different feeding solutions behind a parafilm membrane. Entomol. Exp. & Appl. 8: 107-22. (Calif.)

Naber, E. C. and G. W. Ware. 1965. Effect of Kepone and mirex on reproductive performance in the laying hen. J. Poultry Sci. 44: 875-80. (Ohio)

Naegele, J. A., B. Polcik and J. Nowoslelski. 1965. Daily rhythm of oviposition in the two-spotted spider mite. J. Econ. Entomol. 58: 467-69. (Mass.)

Nakatsugowa, T., and P. A. Dahm. 1965. Parathion activation enzymes in the fat body microsomes of the American cockroach. J. Econ. Entomol. 58: 500-9. (Iowa)

Outenmann, W. H. and D. J. Lisk. 1965. Conversion of 4-(2,4-DB) herbicide to 2,4-D by bluegills. N. Y. Fish and Game J.12: 108-11. (N.Y.C.)

Pennell, J. T. and H. Tsuyuki. 1965. An oxidase-peroxidase system in various insects. Canadian J. of Zool. 43: 587-89. (N.Y.C.)

Philleo, W. W., R. D. Schonbrod and L. C. Terriere. 1965. Methylene-dioxyphenyl) compounds as inhibitors of the hydroxylation of naphthalene in house flies. Agr. and Food Chem. 13: 113. (Oreg.)

Pipa, R. L. and P. S. Woolever. 1965. Insect neurometamorphosis. II. The fine structure of perineural connective tissue, adipohemocytes, and the shortening ventral nerve cord of a moth, Galleria mellonella (L.) Z. Zellforsch. 68: 80-101. (Calif.)

Richards, A. G. 1965. The developmental rate and oxygen consumption of snail eggs at various temperatures. Zts. Naturforsch. 20b: 347-49. (Minn.)

Roemhild, George. 1965. Respiration of eggs and parts of eggs of Aulocara elliotti. Physiol. Zocl. 38: 213-18. (Mont.)

Sherman, M., E. Ross and M. T. Y. Chang. 1965. Acute and subacute toxicity of several insecticides to chicks. Toxicol. & Appl. Pharm. 7: 606-8. (Hawaii)

Shorey, H. H. and L. E. Gaston. 1965. Sex pheromones of noctuid moths. V. Circadian Rhythm of Pheromone-Responsiveness in Males of Autographa californica, Heliothis virescens, Spodoptera exigua and Trichoplusia ni (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Amer. 58: 597. (Calif.)

Shorey, H. H., L. K. Gaston and J. S. Roberts. 1965. Sex pheromones of noctuid moths. VI. Absence of behavioral specificity for the female sex pheromones of Trichoplusia ni versus Autographa californica and Heliothis zea versus H. virescens (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Amer. 58: 600. (Calif.)

Shorey, H. H. and L. K. Gaston. 1965. Sex pheromones of noctuid moths. VII. Quantitative aspects of the production and release of pheromone by females of Trichoplusia ni (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Amer. 58: 604. (Calif.)

Shorey, H. H., and L. K. Gaston. 1965. Sex pheromones of noctuid moths. VIII. Orientation to light by pheromone-stimulated males of Trichoplusia ni (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Amer. 58: 833. (Calif.)

Srivastava, S. C. and A. C. Richards. 1965. An autoradiographic study of the relation between homoctyes and connective tissue in the wax moth, Galleria mellonella L. Biol. Bull., 128: 337-45. (Minn.)

Street, J. C. 1965. Domestic Animals. Research in Pesticides, C. Chichester, ed., Academic Press. N. Y. p. 151-57. (Utah)

Taylor, James F., and Ernest Hodgson. 1965. The origin of phospholipid ethanolamine in the blowfly, Phormia regina (Meig.). J. Insect Physiol. 11: 281. (N.C.)

Taylor, R. I. and A. G. Richards. 1965. Integumentary changes during moulting of arthropods with special reference to the subcuticle and ecdysial membrane. J. Morph. 116: 1-22. (Minn.)

Ting, Kathy Yu and Marion A. Brooks. 1965. Sodium: potassium ratio in insect cell culture and the growth of cockroach cells (Blattariea: Blattidae). Ann. Entomol. Soc. Amer., 58: 197-202. (Minn.)

Yurkiewicz, W. J. 1965. Flight exhaustion studies on the blowfly, Phaenicia sericate using C^{14}-labeled sucrose. Ann. Entomol. Soc. Amer. 58: 766. (Pa.)

AREA NO. 21. FUNDAMENTALS OF INSECT STERILITY

Problem. Basic research on insect sterility is needed to determine if this new approach can be used to control or eradicate destructive insects, thus eliminating the hazards often associated with the application of insecticides to crops and livestock or the high cost of other insect-control measures. The sterile-male technique, involving the use of gamma radiation to produce the sterility, and the release of dominant numbers of laboratory-reared sterilized males, has been utilized successfully to eliminate the screw-worm from the Southeast. Both chemicals and radiation have been employed in the sterile release program to prevent establishment of the Mexican fruit fly in California. The use of a sterilizing chemical in combination with a bait to attract insects already in the environment has tremendous possibilities and may prove more widely useful than release of steriles, because insects need not be reared in the laboratory to outnumber native insects. If a majority of native insects can be attracted and sterilized, thus outnumbering the remaining insects in the population, the same effect may be achieved without the expense of rearing, sterilizing, and releasing sterile males. This field is not necessarily limited to the use of baits containing sterilizing chemicals which insects will eat. The insects might be attracted to a light or an odor and receive a sterilizing dose of chemical through contact. Other approaches include the production of mutations in laboratory strains of insects which would not be lethal in the laboratory but would be lethal in nature. Much additional basic work is needed on the genetics and physiology of reproduction of insect pests, and on the effects of various types of sterilants, in order to determine the possibilities inherent in these new approaches to insect control and whether or not they could be utilized to destroy the many insects of economic importance.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term research program on insect sterility and its application to control and eradication of insect pests. Basic research on the fundamentals of insect sterility is conducted at the Metabolism and Radiation Research Laboratory at Fargo, North Dakota. The research is in cooperation with Crops Research and Animal Husbandry Research Divisions and with the North Dakota Experiment Station. Research on sterility in insects produced by gamma radiation and chemosterilants directed principally toward practical application to control specific insects is also conducted at a number of field laboratories and is discussed under other areas.

The Federal scientific effort devoted to research in this area totals 7.2 professional man-years. Of this total 2.2 is devoted to basic studies on radiation sterilization, 2.5 to effects of mutagenic chemicals on reproduction and heredity, 1.5 to cellular effects from exposure to chemical

mutagens or radiation, and 1.0 to genetics of selected economically important insects.

In addition Federal support of research in this area under a grant provides for 0.5 man-years devoted to effect of radiation on insect behavior and tropisms.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3.0 professional man-years is devoted to studies on the fundamentals of insect sterility at the State stations.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Basic Studies on Radiation Sterilization of Insects.

Studies on the radiation sterilization of the cotton boll weevil are being de-emphasized and shifted to sterilization research on the tobacco budworm, Heliothis virescens (Fabricius), and the cabbage looper, Trichoplusia ni (Hübner). The boll weevil sterilization studies currently center around a screening program for an effective and relatively nontoxic chemosterilant for this insect.

Studies on the reproductive pattern of the female budworm have shown that oviposition begins about 3 to 4 days after emergence and continues during the 14- to 15-day lifespan of the female. Pairs of budworms held in individual cages have averaged 800 eggs during the lifetime of the female. Almost 1,700 eggs have been recorded from a single female and up to 500 eggs have been obtained in a single night. Females irradiated with 35 kr or greater doses as late pupae are unable to lay eggs as adults.

Both the budworm and the cabbage looper were found to be extremely resistant to the induction of sterility by radiation. Extremely high doses of radiation are required. The sterilizing doses for the cabbage looper are above 50 kr.

The tobacco budworm male can be sterilized in the late-pupal stage by 45 to 55 kr of gamma rays. This dose reduces the emergence and lifespan of the moths by about 10 to 20 percent. Tests in which the mating ability of the sterilized male has been studied show that although males are able to produce spermatophores and motile sperm, they mate fewer times during their lifetime (about 1.1 and 4.3 for the treated and untreated males, respectively). Mating-competitiveness tests with ratios of up to three sterile males per untreated male and female show that sterilized males are unable to appreciably depress the egg hatch. Since mature sperm are found in both the late-pupal stage and adults, competitiveness tests were conducted using males which had received 45 kr as 2-day-old adults. These males were sterilized but were unable to reduce egg hatch moderately in competition with untreated males at ratios of up to three sterilized males per untreated male. Further studies are being made to determine if the sperm from treated males

is ineffective when the female has received sperm from an untreated male. This has been observed in the codling moth and might explain the inability of treated males to depress egg hatch effectively, even when they mate and transmit sperm. Untreated females mated to sterilized males lay about half the number of eggs obtained from control crosses.

In future studies we hope to delineate the basic factors for the high radioresistance for these two lepidopteran species. Factors which must be considered are the larval sensitivity, radiation sensitivity in relation to the various stages in spermatogenesis, gonial cell death, and lifespan and activity of irradiated moths.

In a program designed to produce new approaches to insect control, we have studied the factors involved in initiating monogamous response in insect species. Treatments or conditions which prevent mating in insects are theoretically of great potential use in insect control.

A series of experiments involving reproductive-tissue implants, matings with castrate males, and interrupted matings demonstrated that the monogamous reaction in female house flies and stimulus to oviposit are caused by the male seminal fluid rather than sperm or a response to copulation. The monogamous reaction of the female is induced about equally by mating with a castrate male (testes only removed) or by transplanting, into the body cavity of the virgin female, a male copulatory duct. Transplants of testes do not inhibit mating nor does mating with a male from which the copulatory duct has been removed.

The black blow fly, Phormia regina (Meigen), being much larger than the house fly, may be easier to work with in studies involving surgery and the biochemistry of tissues and glands. Therefore, radiation experiments were done which proved that both sexes could be sterilized with as little as 2500 r of gamma rays and that doses as high as 7500 r did not reduce competitiveness of sterilized males. Females mated as readily with sterile males as with normal ones, and would not mate a second time.

The effects of sterilizing doses of radiation on the responses of insects has been investigated by scientists at the University of Georgia under a research grant. Irradiated flies have a different temperature and humidity preference than do nonirradiated flies, but these trends are not always in the same direction and of the same magnitude. Additional studies will be required to determine if these are true differences or variations in the populations. Based on the preliminary results, more detailed experiments are now possible which will be designed for final analysis by IBM computers.

B. Effects of Mutagenic Chemicals on Insect Reproduction and Heredity.

Studies comparing the various methods of administering chemosterilants to boll weevils indicate that feeding low concentrations for a few days in the adult diet is the best method of treatment. Other methods of administration are usually more toxic. Preliminary studies on approximately 100

chemosterilants have shown that 10 of these are effective in reducing the reproductive potential of the boll weevil. Five of these chemosterilants are relatively nontoxic and seem superior to apholate in laboratory cage tests. Additional studies will be conducted in the hope of obtaining still more effective compounds. Studies on the ten selected compounds will be intensified. The major objective in this work is to produce a competitive sterilized male boll weevil. This cannot be accomplished with radiation treatments which severely damage the midgut and reduce the vigor and lifespan of the males. The majority of the chemosterilants studied also produce these effects when administered either topically or orally as a single dose.

The induction of dominant lethal mutations by chemosterilants and the nature of these mutations have been studied in relation to the overall problem of chemically induced insect sterility. When adult male house flies are injected with 1 ul of tretamine, methyltretamine, or the nonalkylating analog, hemel, (at various concentrations) and mated to untreated females, the frequency of dominant lethal mutations found in the first egg batch laid by these females is significantly higher than the frequency of lethals found in the egg batch produced 7 days later. The frequency of lethals induced in the sperm seems to decrease when the sperm are stored in the female. This has been termed "the storage effect." In contrast to this, the sperm of males injected with tepa and four of the phosphine oxide analogs failed to exhibit any change in dominant lethal frequency when stored in the females. This effect is dose and temperature dependent and the maximum effect of the storage reduces the frequency of dominant lethal mutations by more than 50 percent.

The relative efficiency of 11 chemosterilants in inducing dominant lethal mutations has been determined by constructing a dose-response curve for each compound injected into the male house fly. Of the 11 compounds tested, tretamine was found to be the most efficient chemical, producing the maximum amount of dominant lethal mutations at the minimum dose. The least effective chemosterilants were hempa, hemel, and ethyleneimine.

Studies on the mode of action of chemosterilants were conducted to determine whether nonalkylating chemicals are mutagenic. Studies with tepa compared with the effects of hempa showed that both chemicals produced a high proportion of recessive lethal mutations in the sperm of the wasp, Habrobracon, although tepa was far more efficient.

The production of dominant lethal mutations by chemosterilants compared with the production of sperm inactivation by these same chemicals were studied using the parthenogenetic wasp, Habrobracon. It was found that tarsal-contact treatments with residual films with a series of different chemicals induced sterility which was characterized by the induction of dominant lethal mutations in sperm of the insect with no great amounts of sperm inactivation. Some chemosterilants, such as tepa and other phosphine oxide analogs, were found to be highly effective chemosterilants although the type of sterility produced is accompanied by high levels of sperm inactivation. Even doses that are substerilizing produce sperm inactivation, so we are relatively

certain that sperm inactivation by some chemosterilants is not the result of overdosage. The type of sterility produced by various chemosterilants will affect the sterile-male technique of insect control, depending on the monogamous or polygamous nature of the species involved.

C. Cellular Effects in Insects from Exposure to Chemical Mutagens or Radiation.

Spermiogenesis in the boll weevil has been investigated by using tritiated thymidine for radioautography. The results indicate that it takes 10 days for the spermatogonia to mature into functional sperm. Four days are spent in gonial maturation up to meiosis, less than 1 day in meiosis, and the rest in spermiogenesis. This knowledge will serve as a basis for any future study of the cytological effects of chemosterilants.

A method of growing various tissues in vitro has been developed which is reliable for the culture of embryonic insect cells. The process involves the sterilizing and dechorionation of eggs and trypsinization of cells into a cell suspension and then plating the cells in culture bottles. The media currently in use is capable of sustaining the growth for 60 to 90 days.

D. Genetics of Selected Economically Important Insects.

Preliminary studies aimed at developing genetic approaches to insect control have utilized the house fly as the test organism. The number of mutant stocks of house flies possessing genetic markers used in our work has increased from 37 to approximately 75 different stocks during the past year. Much of the research has involved changes in methodology in stock keeping. Both the time and the cost of rearing many different genetic stocks of house flies have been cut approximately in half by changes in techniques. Although a large number of mutants serve as a base for amplified studies on genetic aspects of insect control, the location of these mutant genes on specific chromosomes was not known and had to be determined before more elaborate genetic experiments could be performed. This analysis of the linkage groups to the karyotype relation has been accomplished during the past year. A large number of radiation-induced chromosome translocations (involving about 40 translocation stocks) were described and studied, both genetically and cytologically, to determine which mutant linkage groups were associated with specific chromosomes. With this information available, several experimental crossing schemes have been devised which will assist in the detection of autosomal lethals, semilethals, sex-limited lethals, conditional lethals, or visible mutations. These genetic factors will then be studied for their application to genetic control of insects by the sterile-male technique.

These genetic studies have shown that a large number of mutant stocks contain unknown genetic factors, in addition to the mutant phenotype characteristic of that stock. About half the mutant stocks contain additional, hitherto unreported, phenotypic deviations which will be studied.

PUBLICATIONS--USDA AND COOPERATIVE PROGRAM

Riemann, John G. 1965. The development of eggs of the screw-worm fly, Cochliomyia hominivorax (Coquerel) (Diptera; Calliphoridae) to the blastoderm stage as seen in whole-mount preparations. Biol. Bull. 129(2): 329-39.

PUBLICATIONS--STATE EXPERIMENT STATIONS AND COOPERATIVE PROGRAMS

Hansens, E. J. 1965. Effects of apholate on restricted populations of insecticide resistant house flies, Musca domestica L. J. Econ. Entomol. 58: 944-46. (N.J.)

Roach, S. H. and J. A. Buxton. 1965. Apholate and tepa as chemosterilants of the plum curculio. J. Econ. Entomol. 58: 802-3. (S.C.)

Virkki, N. 1965. Insect gametogenesis as a target. Agr. Sci. Review III (3): 24-37. (P.R.)

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b1(R)	Sugarbeet insect investigations			
ENT b1-1 (R)	Control methods and biological studies of insects and mites affecting sugarbeets	Mesa, Ariz.	No	
		Twin Falls, Idaho	Yes	11-A-1 11-B-1
		Yakima, Wash.	Yes	11-A-1 11-B-1 11-C-1 11-D-1 11-E-1 11-G-1
ENT b2(R)	Tobacco insect investigations			
ENT b2-1 (R)	Biological control methods and biology of insects attacking tobacco foliage	Oxford, N.C.	Yes	10-A-1 10-D-1 10-H-1
		Florence, S.C.	Yes	10-A-1
		Quincy, Fla.	Yes	10-B-3 10-D-1
ENT b2-2 (R)	Insecticide control methods for insects attacking tobacco foliage	Oxford, N.C.	No	
		Quincy, Fla.	Yes	10-B-3 10-C-1
		Florence, S.C.	Yes	10-B-2,3
ENT b2-3 (R)	Control methods and biology of soil insects that attack tobacco	Florence, S.C.	Yes	10-B-1
ENT b2-4 (C)	Attractants, hormones, and sterilization procedures for control of tobacco insects	Lexington, Ky.	No	
		Oxford, N.C.	Yes	10-A-1 10-E-1 10-F-1
		Raleigh, N.C.	No	
		Florence, S.C.	Yes	10-F-3
		Clemson, S.C.	No	
		Quincy, Fla.	Yes	10-E-1,2 10-F-2
		Blacksburg, Va.	No	
		St. Croix, Virgin Islands	Yes	10-E-1
ENT b2-5 (Gr)	Basic studies on the nature and significance of weather as a tool for the prediction and behavior of field populations of insects	Clemson, S.C.	No	
ENT b3(R)	Greenhouse and ornamental plant insects			
ENT b3-1 (R)	Biology and methods of control of insects on greenhouse and ornamental plants	Farmingdale, N.Y.	Yes	12-B-2 12-E-2 12-I-1
		Beltsville, Md.	Yes	12-B-2,3 12-H-1
		Sumner, Wash.	Yes	12-B-4,5 12-E-3
ENT b3-4 1/ (Gr)	Pheromones and reproduction of the bagworm, Thyridopteryx ephemeraeformis (Haw.)	Athens, Ga.	No	
AE-ENT-2 1/ (Gr)	Influence of electromagnetic energy on green peach aphid	Lafayette, Ind.	No	
ENT b4	Vegetable and berry insects			
ENT b4-1 (R)	Biology and methods of control of insects affecting beans	Charleston, S.C.	Yes	1-A-4 1-B-1
		Beltsville, Md.	Yes	1-A-7 1-E-4
		Yakima, Wash.	Yes	1-D-2
		Twin Falls, Idaho	No	
		Riverside, Calif.	No	
ENT b4-3 (R)	Biology and methods of control of insects affecting melons and other cucurbits	Charleston, S.C.	Yes	1-B-2
ENT b4-4 (R2)	Biology and methods of control of the beet leafhopper as a pest of vegetables	Twin Falls, Idaho	No	

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progesss	Line Proj. Incl. in Area & Sub-heading
ENT b4-5 (R)	Insects in relation to diseases of vegetables and berries	Mesa, Ariz.	No	
		Beltsville, Md.	Yes	1-I-1
		Yakima, Wash.	No	
ENT b4-6	Biology, host plant relationships, and methods of control of insects that attack potato	Orono, Me.	Yes	2-A-1 2-B-1 2-D-1 2-H-1 2-I-1
		Yakima, Wash.	Yes	2-B-1,2,3 2-C-1,2,3 2-D-1 2-E-1 2-F-1
ENT b4-7 (R2)	Methods of preventing deleterious residues resulting from the use of insecticides on vegetables and berries	Charleston, S.C.	Yes	1-B-3
		Beltsville, Md.	Yes	1-C-3
ENT b4-8 (R2)	Investigations on the use of natural enemies and other biological methods for the control of vegetable and berry insects	Riverside, Calif.	Yes	1-A-1 1-D-1 1-E-1
		Beltsville, Md.	Yes	1-A-6,7 1-E-5
		Charleston, S. C.	Yes	1-D-1,3,4,5,6,7 1-E-1,2,3
		Yakima, Wash.	Yes	1-D-2
		Mesa, Ariz.	Yes	1-A-1,2 1-D-1
ENT b4-9 (R)	Biology and methods of control of insects and mites affecting strawberries and bramble berries	Beltsville, Md.	No	
		Riverside, Calif.	No	
ENT b4-10 (R)	Biology and methods of insects affecting underground portions of vegetables	Charleston, S. C.	Yes	1-A-5 1-B-4,5,6,7 1-G-1 1-F-1
		Baton Rouge, La.	No	
		Yakima, Wash.	Yes	1-A-3
ENT b4-12 (R)	Improvement of methods and evaluation of equipment for applying insecticides to vegetable crops	Forest Grove, Oreg.	Yes	1-B-10 1-F-3
ENT b4-14 2/	Superseded by ENT b4-20			
ENT b4-16	Control of insects and mites affecting vegetable and berry crops through the development of resistant plant varieties	Yakima, Wash.	Yes	1-H-3
		Beltsville, Md.	Yes	1-H-8
		Charleston, S.C.	Yes	1-H-4,5,6
		Riverside, Calif.	Yes	1-H-1
ENT b4-17 (CA)	Biological control of aphids attacking potatoes	Orono, Me.	Yes	2-D-1
CR-ENT-3 1/ (Gr)	Basic research on insect host-plant interactions involved in plant resistance to the potato leafhopper	Ames, Iowa	No	
ENT-0-0-4 1/ (AID)	Biology, ecology, and development of methods for the control of insect pests of beans, peas, and other vegetable legumes in Asia	Karaj, Iran	No	
ENT b4-18 1/	Basic studies on the biology and behavior of Aphidius spp. and Praon spp. parasites of pea aphid, Acyrthosiphon pisum (Harris)	Walla Walla, Wash.	No	
ENT b4-19 1/ (Gr)	Factors involved in the host association of the two-spotted spider mite	University Park, Pa.	No	

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b4-20 1/	Development of sterilization, attractants, and other specific chemical procedures for control of vegetable insects	Beltsville, Md.	Yes	1-B-8 1-E-6 1-H-7
		Riverside, Calif.	No	
		Mesa, Ariz.	No	
		Charleston, S. C.	No	
CR-ENT-5 1/ (Gr)	Biochemical nature of resistance of strawberries to mites	Lexington, Ky.	No	
ENT b5(R)	Methods of treating plants and commodities regulated by plant quarantines			4-C-2,3
ENT b5-1 (R)	Development of treatments for plants and commodities regulated by plant quarantines	Hoboken, N.J.	Yes	4-G-3
ENT b6	Mexican fruit fly and other fruit pests in Mexico that threaten U. S. horticulture			
ENT b6-1 (R)	Biology, ecology, and methods for control of the Mexican fruit fly and citrus blackfly	Mexico City, Mex.	Yes	4-A-2 4-D-2
ENT b6-2 (R2)	Studies of lures for Mexican fruit flies	Mexico City, Mex.	Yes	4-E-2
ENT b6-4 (R)	Quarantine treatments for Mexican fruit fly infested fruits	Mexico City, Mex.	No	
ENT b7	Investigations of fruit flies in Hawaii			
ENT b7-1 (R)	Ecology and biology of fruit flies and their natural enemies in Hawaii	Honolulu, Hawaii	Yes	4-A-2
		Hilo, Hawaii	No	
ENT b7-2 (R)	Development of new or improved mass production methods and manipulation techniques for fruit flies and their biological control agents	Honolulu, Hawaii	Yes	4-A-2
ENT b7-3 (R)	Investigation of fruit fly lures and repellents in Hawaii	Honolulu, Hawaii	Yes	4-E-2
		Hilo, Hawaii	No	
ENT b7-8	Development of methods for eradication and control of fruit flies in Hawaii	Honolulu, Hawaii	Yes	4-E-2
		Hilo, Hawaii	No	
ENT b7-9	Commodity treatments to destroy fruit flies and associated pests of quarantine importance in fresh fruits and vegetables in Hawaii	Honolulu, Hawaii	Yes	4-G-1
ENT-0-0-3 2/ (AID)	Development of sterilization procedures for the eradication and control of the Mediterranean fruit fly	San Jose, Costa Rica	No	
ENT b8	Deciduous fruit and nut insect investigations			
ENT b8-1 (R2)	Studies of the codling moth and its control	Yakima, Wash	Yes	3-A-1 3-B-1 3-C-1 3-D-1 3-E-1
		Wenatchee, Wash.	Yes	3-E-1
		Kearneysville, W. Va.	Yes	3-B-1
		Vincennes, Ind.	Yes	3-B-1
ENT b8-2 (R2)	Studies of orchard mites and their control	Yakima, Wash.	Yes	3-B-2
		Wenatchee, Wash.	Yes	3-B-2 3-E-5
		Kearneysville, W. Va.	Yes	3-B-2 3-E-5
		Vincennes, Ind.	Yes	3-B-2
ENT b8-3 (R)	Studies of the plum curculio and its control	Ft. Valley, Ga.	Yes	3-A-3
		Vincennes, Ind.	Yes	3-B-4

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b8-4 (R)	Studies of borers attacking deciduous fruit trees and their control	Ft. Valley, Ga.	Yes	3-A-2 3-E-2
		Vincennes, Ind.	Yes	3-A-2 3-A-6 3-D-2 3-E-2
		Kearneysville, W. Va.	Yes	3-B-4
ENT b8-6 (R)	Studies of miscellaneous insect and mite pests of deciduous fruits and their control	Ft. Valley, Ga.	Yes	3-B-4
		Vincennes, Ind.	Yes	3-D-3
		Yakima, Wash.	No	
		Wooster, Ohio	Yes	3-A-6 3-B-4 3-E-5
		Wenatchee, Wash.	Yes	3-B-4
ENT b8-7 (R)	Investigations of nut insects and mites and their control	Albany, Ga.	Yes	3-A-4 3-B-3 3-E-4
		Shreveport, La.	Yes	3-B-3
		Wooster, Ohio	Yes	3-B-4
ENT b8-8 (R)	Grape insect investigations	Wooster, Ohio	No	
ENT b8-10	Ecology, biology, and control of the pear psylla	Yakima, Wash.	Yes	3-B-4
		Wenatchee, Wash.	No	
ENT b8-11 (Gr)	Basic studies on the influence and significance of photoperiod and light on diapause and development of the codling moth	Pullman, Wash.	Yes	3-A-1
ENT b8-12 (Gr)	Basic studies on the nature and significance of chemosterilant and attractant techniques for eradication of the oriental fruit moth	Grand Junction, Colo.	Yes	3-E-5
ENT b8-13 (Gr)	Mass rearing and biology of the peach tree borer (Sanninoidea exitiosa (Say))	Raleigh, N.C.	Yes	3-A-2
ENT b8-14 (Gr)	Basic studies on the nature and significance of sex pheromones and gamma radiation induced sterility of the navel orangeworm, Paramyelois transitella (Walker)	Berkeley, Calif.	Yes	3-E-5
ENT b8-15 1/ (Gr)	A study on the use of chemosterilants alone and in conjunction with established attractants for the control of the apple maggot, Rhagoletis pomonella (Walsh)	Orono, Me.	No	
ENT b8-16 1/ (Gr)	Ecology of mites within pomaceous tree fruit orchards	Provo, Utah	Yes	3-A-6
ENT b8-17 1/ (Gr)	Basic studies on the behavior of the pear psylla (Psylla pyricola (Forster))	Wenatchee, Wash.	Yes	3-A-5
ENT b8-18 1/ (Gr)	Exploratory studies on the use of Ionizing radiation and chemosterilants for the control of the pecan weevil	College Station, Tex.	No	
ENT b8-19 1/ (Gr)	Use of non-diapausing strains of tortricid species in orchard insect control	Geneva, N.Y.	No	
ENT b9(R)	Investigations of insect and mite vectors of deciduous tree fruit viruses			
ENT b9-1 (R)	Distribution of insects and mites in and near deciduous fruit orchards infected with virus diseases	Riverside, Calif.	Yes	3-A-5
ENT b9-2	Studies of insect vectors of phony peach virus disease and their control	Ft. Valley, Ga.	No	

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj.Incl.in Summary of Progress	Line Proj.Incl.in Area & Sub-heading
ENT b9-3 (R)	Studies of mite vectors of peach mosaic virus disease, including biology, ecology, and control	Riverside, Calif.	No	
ENT b9-4 (R)	Transmission studies with possible insect and mite vectors of the latent group of stone fruit viruses	Corvallis, Oreg.	Yes	3-I-2
ENT b9-8	Studies of possible insect and mite vectors of pear decline and their control	Riverside, Calif.	Yes	3-A-5 3-E-3 3-I-1
ENT b9-9	Transmission studies with possible insect and mite vectors of miscellaneous viruses causing diseases of deciduous fruits	Ft. Valley, Ga. Wenatchee, Wash. Corvallis, Oreg.	No No No	
ENT b10	Insects of citrus and other subtropical fruits			
ENT b10-1 (R)	Biology and methods of control of citrus mites	Orlando, Fla. Riverside, Calif.	Yes Yes	4-B-1 4-B-1 4-D-1
ENT b10-2 (R)	Biology and methods of control of scale insects, whiteflies, and mealybugs on citrus	Orlando, Fla. Riverside, Calif. Weslaco, Tex.	No Yes Yes	4-A-1 4-E-1 4-A-1 4-B-1 4-C-1 4-D-1 4-E-1
ENT b10-3 (R)	Biology and methods of control of miscellaneous insects on citrus and other subtropical fruits	Riverside, Calif. Honolulu, Hawaii Mexico City, Mex. Orlando, Fla.	No Yes Yes Yes	4-E-3 4-F-3 4-G-2 4-A-3 4-E-2
ENT b10-4 (R)	Insect vectors of tristeza and other diseases of citrus	Orlando, Fla.	Yes	4-I-1
ENT b10-5 (R2)	Investigations of the biological control of citrus insects and mites	Orlando, Fla. Riverside, Calif. Weslaco, Tex.	Yes Yes Yes	4-D-1 4-D-1 4-D-1
ENT b10-6 1/ (Gr)	Use of supplemental foods to increase populations of mite predators	Riverside, Calif.	No	
ENT b10-7 1/ (Gr)	Ecological study of the southern green stink bug, Nezara viridula (L.), with special emphasis on attractive plants as trap crops or lures	Honolulu, Hawaii	No	
ENT b11	Japanese beetle, European chafer, and related species			
ENT b11-1 (R)	Investigations of methods for controlling the Japanese beetle and eradicating isolated infestations	Moorestown, N.J.	Yes	12-B-1 12-F-1
ENT b11-2 (R)	Development and improvement of treatments to permit movement of nursery stock and farm products under quarantine regulations	Moorestown, N.J.	No	
ENT b11-3 (R)	Development of methods of making biological assays of insecticidal residues in soils	Moorestown, N.J.	No	
ENT b11-4 (R2)	Investigations of survey methods and biological and chemical control of the European chafer	Geneva, N.Y.	Yes	12-B-6 12-C-1 12-E-4 12-G-1

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b11-6	Ecology, biology, and natural control of the Japanese beetle	Moorestown, N.J.	Yes	12-A-1 12-D-1 12-E-1,4
ENT b11-7 (Gr)	Basic studies of the biology of the Cuban May beetle, Phyllephaga bruneri (Chapine)	Gainesville, Fla.	Yes	12-A-2 12-B-7 12-E-5
ENT b11-8 1/	Development of mass production methods for the Japanese beetle, European chafer and related species	Geneva, N.Y. Moorestown, N.J.	No Yes	 12-A-1
ENT c1	Boll weevil investigations			
ENT c1-1 (R)	Biological research on the boll weevil	Florence, S.C. State College, Miss. Stoneville, Miss. Tallulah, La. Waco, Texas Brownsville, Tex. Spur, Texas	Yes Yes Yes Yes Yes Yes Yes	9-A-1 9-A-1 9-A-1 9-A-1 9-A-1 9-A-1 9-A-1
ENT c1-2 (R)(C)	Development of more effective insecticides and formulations and more efficient application methods for control of the boll weevil	Florence, S.C. State College, Miss. State College, Miss. Stoneville, Miss. Tallulah, La. College Station, Tex. Waco, Texas Tucson, Ariz.	Yes Yes Yes Yes Yes Yes Yes Yes	9-B-1 9-E-1 9-B-1 9-E-1 9-B-1 9-B-1 9-B-1 9-B-1
ENT c1-3 (R)	Physiological and nutritional research on the boll weevil	Florence, S.C. State College, Miss. Baton Rouge, La. College Station, Tex.	Yes Yes Yes Yes	9-A-1 9-A-1 9-A-1 9-A-1
ENT c1-4 (C)	Discover and develop methods other than insecticidal for controlling the boll weevil	State College, Miss. State College, Miss. State College, Miss. Baton Rouge, La. Birmingham, Ala.	Yes Yes Yes Yes Yes	9-C-1 9-D-1 9-F-1 9-C-1 9-D-1
ENT c1-5 (C)	Discover and develop methods for eradicating the boll weevil	State College, Miss. Stoneville, Miss. Birmingham, Ala.	Yes Yes Yes	9-D-1 9-D-1 9-D-1
ENT c1-6 (C)	Determine the number of weevils surviving the winter and period of emergence for hibernation quarters in a 4-county area of central Texas	College Station, Tex.	No	
ENT c2	Bollworm investigations			
ENT c2-1 (R)	Biological, physiological and nutritional research on the bollworm and tobacco budworm	Stoneville, Miss. Tallulah, La. Brownsville, Tex. College Station, Tex. Waco, Texas Baton Rouge, La.	Yes Yes Yes Yes Yes Yes	9-A-2 9-A-2 9-A-2 9-A-2 9-A-2 9-A-2

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl.in Summary of Progress	Line Proj. Incl.in Area & Sub-heading
ENT c2-2 (R)	Development of more effective insecticides and formulations and more efficient application methods for control of the bollworm and tobacco budworm	Florence, S.C.	Yes	9-B-2
		Brownsville, Tex.	Yes	9-B-2
		Waco, Texas	Yes	9-B-2
		Tucson, Ariz.	Yes	9-B-2
ENT c2-3 (C)	Discover and develop methods other than insecticidal for controlling the bollworm and tobacco budworm	Florence, S.C.	Yes	9-C-2
		Florence, S.C.	Yes	9-E-2
		Tallulah, La.	Yes	9-C-2
		Waco, Texas	Yes	9-C-2
		Brownsville, Tex.	Yes	9-C-2
		Progreso, Tex.	Yes	9-C-2
		Tucson, Ariz.	Yes	9-C-2
ENT c2-4 (C)	Bionomics of boll weevil and bollworm populations as related to cotton insect control practices	Stoneville, Miss.	Yes	9-C-2
		Rolling Fork, Miss.	Yes	9-C-2
ENT c2-5 (Gr)	The biology and ecology of spiders occurring in cotton fields of the San Joaquin Valley of California and their effects on populations of bollworms, lygus bugs and other cotton pests	Davis, Calif.	Yes	9-C-2
ENT c2-6 1/ (Gr)	Basic studies on the nature and significance of factors affecting the efficiency of Caleomegilla maculata (DeGeer) as a predator of lipidapterous eggs	Fayetteville, Ark.	No	
ENT c3	Cotton insects other than boll weevil, bollworm, and pink bollworm and insects attacking other fiber insects			
ENT c3-1 (R)	Biological, physiological and nutritional research on miscellaneous insect and spider mite pests of cotton	Stoneville, Miss.	Yes	9-A-4
		State College, Miss.	Yes	9-A-4
		College Station, Tex.	Yes	9-A-4
		Waco, Tex.	Yes	9-A-4
		Tucson, Ariz.	Yes	9-A-4
ENT c3-2	Development of more effective insecticides and formulations and more efficient application methods for control of miscellaneous insect and spider mite pests of cotton	Florence, S.C.	Yes	9-B-4
		Stoneville, Miss.	Yes	9-B-4
		Waco, Tex.	Yes	9-B-4
		College Station, Tex.	Yes	9-B-4
		Brownsville, Tex.	Yes	9-B-4
		Tucson, Ariz.	Yes	9-B-4
ENT c3-3	Discover and develop methods other than insecticidal for controlling miscellaneous insect and spider mite pests of cotton	Brownsville, Tex.	Yes	9-C-3
		State College, Miss.	Yes	9-F-2
		Tucson, Ariz.	Yes	9-F-2
		Waco, Tex.	Yes	9-F-2
ENT c3-4 1/ (Gr)	Basic studies on the nature and significance of pathogenic agents of Tetranychus spp. on cotton	Auburn, Ala.	No	
ENT c4	Pink bollworm investigations			
ENT c4-1 (R)	Development of more effective insecticides and formulations and more efficient application methods for control of the pink bollworm	Brownsville, Tex.	Yes	9-B-3
		Tucson, Ariz.	Yes	9-B-3

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c4-8	Biological, physiological, and nutritional research on the pink bollworm	Waco, Tex.	Yes	9-A-3
ENT c4-9	Discover and develop other than insecticidal for controlling or eradicating the pink bollworm	Brownsville, Tex. Brownsville, Tex.	Yes Yes	9-D-2 9-E-3
ENT c5	Corn insects			
ENT c5-1 (R)	Biology and ecology of the European corn borer	Ankeny, Iowa	Yes	7-A-1 7-E-1
ENT c5-2 (R)	Chemical control of the European corn borer	Ankeny, Iowa	Yes	7-B-1
ENT c5-3 (R)	Plant resistance to the European corn borer	Ankeny, Iowa Wooster, Ohio	Yes Yes	7-G-1 7-G-1
ENT c5-4 (R)	Biological control of the European corn borer	Ankeny, Iowa	Yes	7-D-1
ENT c5-5 (R)	Biology, ecology, and methods of control of the corn earworm	State College, Miss. Tifton, Ga.	Yes Yes	7-A-1 1-A-8 1-B-9 1-C-1,2,4,5 1-D-8 1-F-2 1-H-9 7-A-1 7-B-1 7-D-1 7-G-1
ENT c5-6 (R)	Biology, ecology, and methods of controlling miscellaneous insects attacking corn	State College, Miss. Tifton, Ga.	Yes Yes	7-B-1 7-A-1 7-C-1 7-D-1 7-E-1 7-F-1 7-G-1
ENT c5-7 (R)	Plant resistance of corn to rice weevil attack			
ENT c5-8 (R)(C)	Biology, ecology, and methods of control of soil insects attacking corn	Brookings, S. Dak.	Yes	7-A-1 7-B-1 7-F-1 7-G-1
ENT c5-9 (C)	Distribution, biology, ecology, and control of insect vectors of corn diseases	State College, Miss.	Yes	7-H-1
ENT c5-10 1/ (Gr)	Insect transmission of viruses that cause stunting of corn	Columbia, Mo.	Yes	7-H-1
ENT c5-11 1/	Biological control of corn rootworm and other soil insects	Brookings, S. Dak.	No	
ENT c5-12 1/ (CA)	Chemistry of a sex attractant in the southwestern corn borer Zeadiatrea grandiosella (Dyer)	Auburn, Ala.	No	
ENT c5-13 1/ (CA)	Response of insects to radiations in the infrared and microwave regions	Berkeley, Calif.	No	
ENT c5-14 1/ (CA)	Mite transmission of corn viruses	Wooster, Ohio	No	
AE-ENT-1 (C)	Investigation of insect attraction and and communication possibilities in the infrared special region	Dearborn, Mich.	No	

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl.in Summary of Progress	Line Proj. Incl.in Area & Sub-heading
CR-ENT-2 (Gr)	Biochemical basis for resistance of maize to attach by the European corn borer	Ames, Iowa	Yes	7-G-1
ENT-0-0-2 (AID)	Biology, ecology, and development of methods for the control of sorghum, millet, and maize insects in Africa	Zaria, No. Nigeria, and Serere, Uganda, Africa	No	
ENT c6	Small grain insects			
ENT c6-1 (R)	Biology, ecology, and methods of control of aphids attacking small grains	Stillwater, Okla.	Yes	7-G-2
		Brookings, S. Dak.	Yes	7-A-2 7-G-2
ENT c6-3 (R)	Biology, ecology, and methods of control of Hessian fly and wheat jointworm attacking small grains	Tifton, Ga.	No	
		Manhattan, Kans.	No	
		West Lafayette, Ind.	Yes	7-A-2
ENT c6-4 (R)	Biology, ecology, and methods of control of the wheat stem sawfly	Bozeman, Mont.	Yes	7-D-2
		Fargo, N.Dak.	Yes	7-A-2 7-D-2 7-E-2 7-G-2
ENT c6-5 (R)	Biology, ecology, and methods of control of insects attacking sorghums	Stillwater, Okla.	Yes	7-A-2 7-B-2
		Tifton, Ga.	Yes	7-G-2
ENT c6-6 (R)	Biology, ecology, and methods of control of soil insects and related pests of small grains	Brookings, S. Dak.	Yes	7-E-2
ENT c6-7 (R)	Distribution, biology, ecology, and control of insect and mite vectors of small grain diseases	Brookings, S. Dak.	Yes	7-H-2 8-H-1 8-B-1
ENT c6-8	Biology, ecology, and methods of control of rice field insects	Baton Rouge, La.	Yes	8-A-1 8-B-1 8-C-1 8-G-1
ENT c6-9 (C)	Biology, ecology, and methods of control of Oulema melanopa attacking small grains	East Lansing, Mich.	Yes	7-A-2 7-D-2 7-E-2 7-G-2
ENT c6-10 (Gr)	Behavior of cereal leaf beetle as affected by climatic factors	Lafayette, Ind.	Yes	7-A-2
ENT c6-11 (Gr)	Microbiology and pathologies of Oulema melanopa (L.)	Columbus, Ohio	Yes	7-E-1
ENT c6-12 (Gr)	Control of damage by larvae of the rice water weevil (Lissorhopterus oryzophilus Kuschel) by increasing plant tolerance	Fayetteville, Ark.	Yes	8-G-1
ENT c7	Sugarcane insects			
ENT c7-1 (R)	Biology, ecology, and methods of control of borers attacking sugarcane	Houma, La.	Yes	11-A-2 11-B-2 11-D-2 11-E-2 11-F-1
		Canal Point, Fla.	Yes	11-A-2
ENT c7-2 (R)	Biology, ecology, and methods of control of insects other than borers attacking sugarcane	Houma, La.	Yes	11-A-2
		Canal Point, Fla.	Yes	11-D-2
ENT c7-3 (R)	Biology, ecology, and methods of control of insect and mite vectors of sugarcane diseases.	Houma, La.	Yes	11-G-2
ENT c7-4 (Gr)	Factors affecting the efficiency of Trichogramma spp. as parasites of lepidopterous pests	Baton Rouge, La.	Yes	11-D-2

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl.in Summary of Progress	Line Proj. Incl.in Area & Sub-heading
ENT c8	Legume and grass insects			
ENT c8-2 (R)	Biology, ecology, and methods of control of insects attacking legumes other than alfalfa and clovers	Columbia, Mo.	Yes	6-A-1 6-B-2 6-C-1 6-D-1
		Tifton, Ga.	Yes	6-A-2 6-B-1 6-C-2
ENT c8-3 (R)	Biology, ecology, and methods of control of insects attacking grasses	Lincoln, Nebr.	Yes	5-A-4
		Tifton, Ga.	Yes	5-A-4 5-B-4 5-E-1,2 5-G-8
		Forest Grove, Oreg.	Yes	5-A-4
		University Park, Pa.	Yes	5-D-8
ENT c8-4 (R)	Insect vectors of pathogenic agents affecting legumes and grasses	University Park, Pa.	Yes	5-H-1,2
		Columbia, Mo.	Yes	6-D-1
ENT c8-5 (R)	Insecticide residues on forage crops	Tifton, Ga.	Yes	5-C-1,2,3
		Beltsville, Md.	Yes	5-C-1,2,3
		Bozeman, Mont.	Yes	5-C-4
		Yakima, Wash.	Yes	5-C-4
ENT c8-6 (C)	Biology, ecology, and methods of control of aphids, leafhoppers, seed chalcids, and miscellaneous insects attacking alfalfa	Lincoln, Nebr.	Yes	5-A-2 5-B-2 5-G-1
		Tucson, Ariz.	Yes	5-A-2 5-D-3 5-G-3,5
		Mesa, Ariz.	Yes	5-B-2 5-G-4
		University Park, Pa.	Yes	5-G-1
		Lincoln, Nebr.	Yes	5-G-3
ENT c8-7	Biology, ecology, and methods of control of insects attacking clover and sweetclover	Lincoln, Nebr.	Yes	5-A-3 5-B-3 5-E-3 5-G-6,7
		University Park, Pa.	Yes	5-H-1,2
ENT c8-8	Biology, ecology, and methods of control of the alfalfa weevil	Beltsville, Md.	Yes	5-A-2 5-B-2 5-D-2 5-F-1 5-G-2
		Lincoln, Nebr.	Yes	5-D-2
ENT c8-9 (C)	Mass production and distribution of Neodusmetia sangwani, a parasite of the Rhodesgrass scale	Weslaco, Tex.	Yes	5-D-6
ENT c8-10 (Gr)	Ovipositional behavior of the alfalfa seed chalcid to chemicals occurring naturally in alfalfa	Laramie, Wyo.	Yes	5-E-5
ENT c8-11 (Gr)	Attractants and stimulants for the alfalfa weevil	Blacksburg, Va.	Yes	5-E-4
ENT c8-12 (Gr)	Resistance of alfalfa plants and varieties to tarnished plant bug and other mirid species (lygus bugs)	Manhattan, Kans.	Yes	5-G-5

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Sub-heading
ENT c8-14 1/ (Gr)	Testing for genetic resistance to thrips in peanuts	Stillwater, Okla	Yes	6-C-2
ENT c8-15 1/ (Gr)	Physiology of injury caused by lygus bugs	Davis, Calif.	Yes	5-A-2
CR-ENT-1 (Gr)	Nature of resistance of Melilotus infesta to sweetclover weevil	Lincoln, Nebr.	Yes	5-G-7
ENT c9	General feeder insects			
ENT c9-1 (R)	Biology, ecology, and biological methods of control of armyworms and cutworms	Baton Rouge, La.	Yes	5-D-5
ENC c9-2 (R)	Biology, ecology, and methods of control of grasshoppers	Mesa, Ariz.	Yes	5-A-1
		Bozeman, Mont.	Yes	5-B-1
				5-D-1
ENT c9-3 (R)	Biology, ecology, and methods of control of white-fringed beetles	Gulfport, Miss.	Yes	5-A-5
				5-B-5
				5-D-7
ENT c9-4 (Gr)	The development of artificial rearing techniques for the white-fringed beetle	Auburn, Ala.	Yes	5-A-5
ENT c9-5 1/ (Gr)	A study of viruses in grasshoppers	Bozeman, Mont.	Yes	5-D-1
ENT c9-6 1/ (Gr)	Food habits of selected Great Plains grasshoppers inhabiting cultivated grasslands versus rangelands	Manhattan, Kans.	Yes	5-A-1
ENT c10	Bee culture investigations			
ENT c10-1 (R)	Etiology of bee diseases and development of control methods for diseases and pests	Baton Rouge, La.	Yes	18-C-1,2,13
		Beltsville, Md.	Yes	18-C-1,3,4, 5,6,7,8
		Laramie, Wyo.	Yes	18-C-1,3,4, 7,9,10, 11,12
		Tucson, Ariz.	Yes	18-C-1
		Madison, Wisc.	Yes	18-C-1,7
ENT c10-2 (R)	Biology and breeding for improvement of the honey bee	Baton Rouge, La.	Yes	18-A-3
		Madison, Wisc.	Yes	18-A-1,2
		Logan, Utah	Yes	18-A-3
		Beltsville, Md.	Yes	18-A-4
ENT c10-3 (R)	Behavior and utilization of honey bees in the pollination of agricultural and other economic crops	Tucson, Ariz.	Yes	18-D-4,6,7, 8,9
		Madison, Wisc.	Yes	18-D-1,2,3
		Logan, Utah	Yes	18-D-5
ENT c10-4 (R)	Biology and utilization of insects other than honey bees in the pollination of agricultural crops	Logan, Utah	Yes	18-F-1,2
ENT c10-5 (R)	Effect of pesticides, insect diseases, and farm practices on honey bees and other pollinating insects	Logan, Utah	Yes	18-E-6
		Baton Rouge, La.	Yes	18-E-2
		Laramie, Wyo.	Yes	18-E-4
		Madison, Wisc.	Yes	18-E-1
		Tucson, Ariz.	Yes	18-E-3
		Beltsville, Md.	Yes	18-E-5

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c10-6	Management for improvement in productivity of honey bees	Madison, Wisc.	Yes	18-B-1,2,3, 4,5
		Logan, Utah	Yes	18-B-7-8
		Beltsville, Md.	Yes	18-B-3
		Tucson, Ariz.	Yes	18-B-6
ENT c10-7 (Gr)	Pathogenesis and diagnosis of Nosema disease in Apis mellifera L.	Columbus, Ohio	No	
ENT c10-8 1/ (Gr)	Glands in bees, their topography, innervation, morphology, histology and physiology	Logan, Utah	No	
ENT c10-9 1/ (Gr)	A behavorial study of the effect of hormonal secretions of the queen honey bee (Apis mellifera L.) on the industriousness of worker honey bees	Urbana, Ill.	No	
ENT h1	Mosquitoes, sand flies, and gnats investigations			
ENT h1-1 2/	Superseded by ENT h1-24			
ENT h1-4	Studies on the distribution, abundance, taxonomy, and biology of mosquitoes affecting agriculture	Gainesville, Fla.	Yes	13-A-1 13-C-1 17-A-1
		Corvallis, Oreg.	Yes	13-A-1 13-C-1 17-A-1
		Fresno, Calif.	No	
		Lake Charles, La.	Yes	13-A-1 13-C-1 17-A-1
ENT h1-5 2/	Superseded by ENT h1-25			
ENT h1-15 (R2)	Studies on the relationship of water and land management procedures to mosquito breeding in water impoundments and irrigated farming areas	Fresno, Calif. Corvallis, Oreg.	No No	
ENT h1-16 2/	Superseded by ENT h1-23			
ENT h1-17 (C)	Studies on the biology and control of salt-marsh and ricefield mosquitoes in Louisiana and other Gulf Coast areas	Lake Charles, La.	Yes	13-A-1 17-A-1
		Lafayette, La.	Yes	13-A-1 17-A-1
ENT h1-18 (C)	The effect of predators and parasites on the breeding potential of mosquitoes found in coastal-marsh areas of Louisiana	Lake Charles, La.	Yes	13-C-1 17-C-1
ENT h1-19 (Gr)	Population dynamics, sterilization, and attractants for the eye gnat, Hippelates pusio Loew	Gainesville, Fla.	Yes	17-A-3
ENT h1-20 (C)	Dispersal of concentrated or undiluted insecticides for increased effectiveness and economy in mosquito control	Berkeley, Calif.	Yes	13-B-1 17-B-1
ENT h1-21 (C)	Pathogens as biological control agents for mosquito larvae	Berkeley, Calif.	Yes	13-C-1 17-C-1
ENT h1-22 1/ (Gr)	Behavior and food preferences of introduced annual fishes in relation to mosquitoes	Riverside, Calif.	No	
ENT h1-23 1/	Biology and control of black flies, sand flies, and other gnats and their relationship to disease transmission, especially on livestock and poultry	Denver, Colo. Gainesville, Fla.	Yes No	13-G-2

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl.in Area & Sub-heading
ENT h1-24 [1]	Development of effective insecticides and other materials and methods for controlling mosquitoes.	Gainesville, Fla.	Yes	13-B-1 13-C-1 13-D-1 17-B-1 17-C-1 17-D-1
		Corvallis, Oreg.	Yes	13-B-1 13-C-1 17-B-1 17-C-1
		Fresno, Calif.	No	
		Lake Charles, La.	Yes	13-C-1 17-C-1
ENT h1-25 [1]	Development of repellents and other materials and improved methods to protect man and animals from mosquitoes, sand flies, and gnats	Gainesville, Fla.	Yes	17-F-1
		Corvallis, Oreg.	Yes	17-F-1
ENT h1-26 [1] (Gr)	Host animals of mosquitoes and other biting Diptera in certain areas of Louisiana	Baton Rogue, La.	No	
ENT h1-27 [1] (Gr)	Biology, ecology, and methods of rearing several species of Culicoides found in Virginia	Blacksburg, Va.	No	
ENT h1-28 [1] (Ca)	Studies on insecticide resistance and the sterility principle of control for insect vectors of diseases	Gainesville, Fla.	No	
ENT h2	Investigations on flies affecting man and livestock			
ENT h2-1 [2]	Superseded by ENT h2-23			
ENT h2-5 [2]	Superseded by ENT h2-24			
ENT h2-7 [2]	Studies of irradiation and radio-active insecticides on flies and other arthropods affecting man and animals (Discontinued)			
ENT h2-9 [2]	Superseded by ENT h2-25			
ENT h2-11 [2]	Superseded by ENT h2-26			
ENT h2-14 (R2)	Development of repellents and other methods to protect man from horse flies, deer flies, and stable flies.	Gainesville, Fla.	No	
ENT h2-15 (R2)	Development of improved media and mass rearing and distribution techniques for screw-worm control	Mission, Tex.	Yes	13-A-4
ENT h2-16 (R2)	Development of attractants and other materials and methods for estimating and controlling natural screw-worm populations	Mission, Tex.	Yes	13-A-4 13-D-3 13-F-1
ENT h2-17 (R)	Development of physical and mechanical methods of controlling flies and other pests of livestock	Beltsville, Md.	Yes	13-B-2 17-B-2
ENT h2-18 (C)	Insecticidal methods for controlling the dog fly (Stomoxys calcitrans (Linnaeus)) in the Gulf Coast area of Northwestern Florida	Panama City, Fla.	Yes	13-B-4 17-B-4
ENT h2-19 (Gr)	Effect of predacious mites in reducing fly production from poultry droppings	Berkeley, Calif.	Yes	13-C-2 17-C-2
ENT h2-20 (Gr)	Basic biology and behavior of Tabanids (horse flies)	Laramie, Wyo.	Yes	13-A-3
ENT h2-21 [1] (Gr)	Response of the horn fly to extracts of animal tissues and to putrefaction products	Las Cruces, New Mexico	No	
ENT h2-22 [1] (Gr)	The biology and control of Tabanidae in marsh-lands along the Eastern shore of the Great Salt Lake, Utah	Salt Lake City, Utah	No	

Line Project Check List-- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl.in Area & Sub-heading
ENT h2-23[1/]	Insecticides, repellents, and other materials and methods for the control of horn flies, stable flies, and face flies	Kerrville, Tex.	Yes	13-A-5,8 13-B-4,6 13-D-4,5
		Corvallis, Oreg.	Yes	13-A-5,6 13-B-6
		Lincoln, Neb.	Yes	13-B-7 13-C-4
		Stoneville, Miss.	Yes	13-B-6
		Beltsville, Md.	Yes	13-B-2
		Gainesville, Fla.	Yes	13-B-4 17-B-3
ENT h2-24[1/]	Development of improved larvicides and other materials and methods for the control of screw-worms and fleeceworms	Mission, Tex.	Yes	13-B-3 17-B-3
ENT h2-25[1/]	Development of insecticides, chemosterilants, attractants, biological agents, and other materials and methods for the control of house flies and blow flies	Gainesville, Fla.	Yes	13-B-2 13-D-2 17-B-2 17-D-2 17-E-2 17-F-2
		Corvallis, Oreg.	Yes	13-A-2 13-B-2 13-D-2 17-A-2 17-B-2 17-D-2 17-E-2 17-F-2
		Kerrville, Tex.	Yes	17-B-5
		Lincoln, Neb.	No	
ENT h2-26[1/]	Studies on the biology and control of horse flies and deer flies as they relate to pests of animals and vectors of disease	Stoneville, Miss.	Yes	13-A-3
		Corvallis, Oreg.	No	
		Kerrville, Tex.	Yes	13-A-3
ENT h2-27[1/] (Gr)	The influence of native parasites on the population levels of face flies and other dung-breeding Diptera	Blacksburg, Va.	No	
ENT h2-28[1/] (Gr)	Regulation of feeding in selected insects	Raleigh, N.C.	No	
ENT-0-0-1 (AID) (R)	Studies on the biology and control of tsetse flies in Africa	Salisbury, Rhodesia Africa	Yes	13-D-6 17-D-4
ENT h3(R)	Cattle grub and bot fly investigations			
ENT h3-1[2/]	Superseded by ENT h3-3			
ENT h3-2[1/] (Gr)	Basic studies on the mode of action of systemic insecticides applied to sheep for sheep nose bot control	Lexington, Kentucky	No	
ENT h3-3[2/]	Development of new insecticides and other materials and methods for the control of grubs and bots affecting livestock	Kerrville, Tex.	Yes	13-A-7 13-B-5
		Corvallis, Oreg.	Yes	13-A-7 13-B-5
ENT h4	Lice, mites, ticks, and fleas affecting man and animals investigations			
ENT h4-1[2/]	Superseded by ENT h4-15			
ENT h4-3[2/]	Superseded by ENT h4-16			
ENT h4-7 (R2)	Development of insecticides and other methods for the control of human lice and itch mites affecting man	Gainesville, Fla.	Yes	17-B-5
ENT h4-8 (R2)	Development of insecticides and other methods for the area control of ticks, mites, and fleas with particular reference to protecting man	Gainesville, Fla.	Yes	17-B-6

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h4-9 (R2)	Development of repellents and other methods to protect man from mites, ticks, and fleas	Gainesville, Fla.	Yes	17-E-4
ENT h4-10 (R2)	Development of insecticides and other materials and methods for the control of ticks and sheep ked on animals	Kerrville, Tex. Corvallis, Oreg.	Yes Yes	13-A-9 13-B-8
ENT h4-11 (R)	Studies on the role of ticks, mites, lice, fleas, and other arthropods in the transmission of diseases of livestock and poultry	Stoneville, Miss. Beltsville, Md.	Yes Yes	13-G-1 13-G-1
ENT h4-12	Studies on the role of ticks and other arthropods in the transmission of equine piroplasmosis and on the development of insecticides and other means of controlling or eradicating vectors of the disease	Kerrville, Tex. Beltsville, Md.	No Yes	 13-G-2
ENT h4-13 (Gr)	Biological studies on the mite Neoschongastia americana (Hirst) on turkeys in Georgia	Athens, Ga.	Yes	13-A-10
ENT h4-14 1/ (Gr)	Effects of chemosterilants on the northern fowl mite	State College, Miss.	No	
ENT h4-15 1/	Development of improved insecticides and other materials and methods for the control of lice affecting livestock	Stoneville, Miss. Kerrville, Tex. Lincoln, Neb.	No No	 13-B-12
ENT h4-16 1/	Development of improved materials and methods for the control of external parasites of poultry	Corvallis, Oreg. Kerrville, Tex.		13-B-10 13-B-9 13-B-11
ENT h7	Toxicity and residue studies on insecticides and repellents in relation to the control of insects affecting livestock			
ENT h7-1 2/	Superseded by ENT h7-5			
ENT h7-2 (R2)	Extent of storage of insecticides in animal tissues and amount secreted in milk of dairy cattle when used for insect control	Kerrville, Tex.	Yes	13-E-1
ENT h7-4 (R2)	Develop quantitative bioassay methods for analysis of insecticidal chemical residues	Kerrville, Tex.	No	
ENT h7-5 1/	Investigations relating to the acute and chronic toxicity of insecticides, repellents, and other materials to livestock	Kerrville, Tex.	Yes	13-E-2
ENT h10	Household insect investigations			
ENT h10-1 (R2)	Development of measures for the control of insects in homes	Gainesville, Fla.	Yes	17-B-4 17-B-7 17-D-3 17-E-3
ENT j1	Identification and classification of insects			
ENT j1-1 (R2)	Identification and classification of hemipterous insects	Washington, D. C.	Yes	20-A-6,7 20-C-5
ENT j1-2 (R2)(C)	Identification and classification of beetles	Washington, D. C. College Station, Tex.	Yes Yes	20-A-5 20-C-6
ENT j1-3 (R2)	Identification and classification of moths and butterflies	Washington, D. C.	Yes	20-C-4
ENT j1-4 (R2)	Identification and classification of grasshoppers and allied insects	Washington, D. C.	Yes	20-A-1
ENT j1-5 (R2)	Identification and classification of two-winged flies	Washington, D. C.	Yes	20-A-3 20-C-1
ENT j1-6 (R2)	Identification and classification of thrips	Washington, D. C.	Yes	20-A-4
ENT j1-7 (R2)	Identification and classification of hymenopterous insects	Washington, D. C.	Yes	20-C-2,3
ENT j1-8 (R2)	Identification and classification of mites, chiggers, and ticks	Washington, D. C.	Yes	20-C-7
ENT j1-11 (Gr)	Basic studies on the taxonomy, morphology, and ecology of cutworm larvae	Ithaca, N. Y.	Yes	20-A-8

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl.in Summary of Progress	Area & Sub-heading
ENT j1-12 (Gr)	Basic studies of the nature and taxonomic significance of morphological characters of females of leafhoppers	Raleigh, N.C.	Yes	20-A-7
ENT j1-13[1/] (Gr)	Basic studies on the morphology of insect receptors stimulated by attractants	New Brunswick, N. J.	No	
ENT j2	Utilization of insect enemies in the control of insect pests and weeds			
ENT j2-6	Biological control of weeds	Albany, Calif.	Yes	21-C-5,13, 14 21-D-3
ENT j2-7 (R)	Search for and importation of foreign parasites and predators of insect pests	Paris, France	Yes	21-A-1 21-C-1,2, 4,7,8,9
ENT j2-8 (R)	Search for and importation of foreign insect enemies of weeds	Rome, Italy	Yes	21-B-1,2, 3,4
		Buenos Aires, Argentina	Yes	21-C-6
ENT j2-9 (C)	Receipt and distribution of foreign natural enemies of insect pests and weeds	Moorestown, N.J.	Yes	21-D-1,2, 3,4
		Albany, Calif.	Yes	21-D-3,4
	Investigation on the micro-organisma which attack Oulema species in Europe and the propagation and release of European insect parasites of Oulema species in Indiana	Lafayette, Ind.	Yes	21-D-4
ENT j2-10 (C)	A study of the insects that feed on range-land weeds of foreign origin in the State of Idaho	Moscow, Idaho	Yes	21-C-14
ENT j2-11 (Gr)	The biologies and host relationships of tachinid parasites of insects in the State of Washington	Pullman, Wash.	Yes	21-C-10
ENT j2-12 (C)	A world review of parasites, predators, and pathogens introduced into new habitats against injurious insects and weeds	Riverside, Calif.	No	
ENT j2-13 (Gr)	Selection and development of superior strains of predators and parasites	Columbia, Mo.	No	
ENT j2-14 (Gr)	Studies on the significance of the life history and ecology of Lebia analis Dej., and important predaceous ground beetle	Fayetteville, Ark.	Yes	21-C-12
ENT j2-15 (Gr)	Insects associated with aquatic weed pests of foreign origin in Louisiana	Baton Rouge, La.	Yes	21-C-5,13
ENT j2-16 (Gr)	The attraction and concentration of insect predators by non-toxic chemical stimuli	St. Paul, Minn.	Yes	21-C-11
ENT j2-17[1/] (Gr)	Studies on the host specificity of a hymenopterous parasite of Lygus (Hemiptera)	Storrs,Conn.	No	
ENT j2-18[1/] (C)	An annotated bibliography and host catalog of North American Tachinidae	San Francisco, Calf.	No No	
ENT j2-19[1/]	Basic studies on the nature and significance of the effectiveness of the parasitic wasp Tetrastichus incertus in controlling infestations of the alfalfa weevil	Ithaca, N.Y.		
ENT m1	Chemical investigations of products of natural origin for insect control			
ENT m1-14 (R)	Investigations of plants as sources of insecticides, synergists, insect repellents or attractants, or antimetabolites	Beltsville, Md. State College, Miss.	Yes Yes	19-A-2 19-A-2
ENT m1-15 (R) (C)	Investigation of substances naturally occurring in insects that might be used to upset their development or reproduction or otherwise affect their vital processes	Beltsville, Md. Yakima, Wash.	Yes Yes	19-A-1 19-A-1
ENT m1-16 (Gr)	Isolation, purification, and characterization of the sex attractant for the tobacco budworm, Heliothis virescens	Ann Arbor, Mich.	Yes	19-A-1

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj.Inc.in Summary of Progress	Line Proj.Inc.in Area & Sub-heading
ENT m1-17 (Gr)	Investigation of the tobacco hornworm sex attractant	Madison, Wis.	Yes	19-A-1
ENT m2	Chemical investigations to develop synthetic organic materials for insect control			
ENT m2-1 2/	Superseded by ENT m2-19			
ENT m2-4 2/	Superseded by ENT m2-21			
ENT m2-13 (R 2)	Chemical investigations of radioactively labeled insect control agents	Beltsville, Md. State College, Miss.	Yes Yes	19-B-1, E-2 19-B-1
ENT m2-15 (R)	Preparation of synthetic organic compounds for testing as insect control or eradication agents through effects other than death	Beltsville, Md. Gainesville, Fla.	Yes Yes	19-B-1 19-B-2
ENT m2-16	Preparation of compounds for testing as insect chemosterilants	Beltsville, Md.	Yes	19-B-1
ENT m2-17	Development of basic chemical information on insect chemosterilants	Beltsville, Md. State College, Miss. Gainesville, Fla.	Yes Yes Yes	19-B-1 19-B-1 19-B-1
ENT m2-18 (C)	Synthesis of organic compounds for use in investigations on insect attractants and chemosterilants	Kansas City, Mo.	Yes	19-B-1 19-B-2
ENT m2-19 1/	Preparation of synthetic organic compounds for evaluation as insecticides and synergists	Beltsville, Md. Gainesville, Fla.	Yes Yes	19-B-2 19-B-2
ENT m2-20 1/ (C)	Determination of toxicological properties of materials under investigation as chemosterilants, attractants, or other new types of insect control agents	Falls Church, Va.	No	
ENT m2-21 1/	Development of formulations of materials for insect control	Beltsville, Md. Gainesville, Fla. College Station, Tex.	Yes Yes Yes	19-B-3 19-B-3 19-B-3
ENT m3	Analysis of pesticides, pesticide residues, and accessory materials			
ENT m3-5	Analysis of insect control chemicals, their formulations, and accessory materials	Beltsville, Md.	Yes	19-B-2 19-C
ENT m3-6 (C)	Determination of residues of insect control chemicals in plant and animal products and in soils	Beltsville, Md. Tifton, Ga. Kerrville, Tex. Yakima, Wash.	Yes Yes Yes Yes	1-C-2,3 2-C-1 10-C-1 1-C-1,4,5 5-C-1,2,3 7-C-1 13-E-1 2-C-1,2,3,4,5 3-C-1 5-C-4 11-C-1
ENT m4	Chemical investigations on fumigants and aerosols for control of insect pests			13-E-1
ENT m4-1 (R2)	Development of formulas and dispensing equipment for aerosols to control insects	Beltsville, Md.	Yes	19-D
ENT m9	Laboratory tests to determine the effectiveness of insect control materials			
ENT m9-1 (R2)	Comparison of the toxic, attractant, arrestant, and repellent action of chemical materials to test insects	Beltsville, Md. Brownsville, Tex.	Yes Yes	19-E-1 19-E-1
ENT m9-3 (R2)	Comparison of insecticidal materials in gas-propelled aerosols and space sprays	Beltsville, Md.	Yes	19-E-3
ENT m9-4 (R)	Biological evaluation and biochemical studies of materials for insect control through effects other than death	Beltsville, Md. Brownsville, Tex.	Yes Yes	19-E-1,2 19-E-1

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Inc. in Summary of Progress	Line Proj. Inc. in Area & Sub-heading
ENT m10	Methods for disinsectization of aircraft (not divided into line projects)	Beltsville, Md.	Yes	19-F
ENT m11	Development of methods of analysis for pesticides and pesticide residues			
ENT m11-2 (R2)	Development of methods of analysis for insect-control chemicals	Beltsville, Md. Tifton, Ga. Kerrville, Tex. Yakima, Wash. College Station, Tex.	Yes Yes Yes Yes Yes	19-C 19-C 19-C 19-C 19-C 19-B-3
ENT P 1	Insect Pathology Laboratory	Beltsville, Md.	Yes	22-A-1,2,3, 4,5,6 22-B-1,2,3, 4,5 22-C-1,2 22-D 22-E-1 22-F-1,2
ENT P 2	Insect Physiology Laboratory	Beltsville, Md.	Yes	23-A-1,2,3 23-B-1,2,3,4 23-C-1
ENT q1	Insect metabolism and physiology			
ENT q1-1	Studies on the metabolism of insecticides and other compounds in insects	Fargo, N. Dak.	Yes	23-D-1
ENT q1-2	Studies on the physiological processes specific to insects	Fargo, N. Dak.	Yes	23-E-1,2,3,4
ENT q1-3	Physiological studies on insect growth and development	Fargo, N. Dak.	Yes	23-F-1,2
ENT q2	Radiation biology and insect genetics			
ENT q2-1	Basic studies on radiation sterilization of insects	Fargo, N. Dak.	Yes	24-A
ENT q2-2	Effects of mutagenetic chemicals on insect reproduction and heredity	Fargo, N. Dak.	Yes	24-B
ENT q2-3	Cellular effects in insects resulting from exposure to chemical mutagens or radiation	Fargo, N. Dak.	Yes	24-C
ENT q2-4	Genetics of selected economically important insects	Fargo, N. Dak.	Yes	24-D
ENT q2-5 (Gr)	Investigations of changes in insect behavior and tropisms resulting from sterilizing doses of radiation	Athens, Ga.	No	
ENT r1	Investigations of insect diseases			
ENT r1-1 (C)	Evaluation of vertebrate toxicity and pathogenicity of insect virus diseases	Chicago, Ill.	No	
ENT r1-2 (C)	Development of methods for the continuous production of virus-susceptible insects and virus disease organisms	College Park, Md. New Brunswick, N.J. Columbus, Ohio	No No No	
ENT r1-3 1/ (C)	Feasibility of growing nuclear polyhedrosis virus from Heliothis zea in bacterial cells or protoplasts	Kansas City, Mo.	No	
ENT r1-4 1/	Methods of mass-producing nuclear polyhedrosis virus-infected populations of the beet armyworm and yellow-striped armyworm, and selection of virulent mutants of nuclear polyhedrosis virus disease of corn earworm	Buena Park, Calif.	No	

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT rl-5 1/	Determination of the virulence, pathogenicity, and interrelations between two species of viruses coexisting in the cabbage looper	Geneva, N.Y.	No	
	P. L 480 Projects			
A6-ENT-4	Biological control of citrus, tobacco, and vegetable aphids	Taiwan	Yes	21-A-4
A7-ENT-1	Investigations of parasites, predators, and pathogens of sugarcane borers in India	India	Yes	21-C-19
A7-ENT-2	Survey of beneficial parasites and predators of agricultural and horticultural crops in the Indian Union	India	Yes	21-A-30
A7-ENT-6	Nutritional studies on the silkworm Bombyx mori L. - its requirements for vitamins and amino acids and its nutrition in relation to the mineral nutrition of its host plant, mulberry (Morus indica) and studies on the host specificity of the silkworm Bombyx mori L.	India	No	
A7-ENT-7	Survey for natural enemies of witchweed, and of water hyacinth and other weeds affecting waterways in India	India	Yes	21-B-4
A7-ENT-8	Developing methods for large-scale rearing of parasites under laboratory conditions	India	Yes	21-C-18
A7-ENT-9	Investigations of parasites, predators, and pathogens of the European corn borer and Heliothis spp. in India	India	Yes	21-C-3
A7-ENT-10	Acarine disease problem of honey bees	India	Yes	18-C-14
A7-ENT-14	Studies on the free amino acids of insect haemolymph and the accumulation of citric acids in insect tissue	India	No	
A7-ENT-17	Control of the coconut rhinoceros beetle Oryctes rhinoceros L.	India	Yes	21-A-5
A7-ENT-19	Biology, ecology, and utilization of insects other than honey bees in the pollination of agricultural crops	India	Yes	18-C-4
A7-ENT-20	Studies of microbiology and pathology of insect pests of crop plants	India	No	
A7-ENT-22 1/	Studies of Indian Jassidae with particular reference to Circulifer and related genera and their importance as vectors of plant virus diseases	India	No	
A7-ENT-24	Systematic and biological studies of Indian thrips	India	Yes	20-A-4
A7-ENT-25	Research on insect pests of maize with special reference to stalk borers	India	Yes	7-G-1
A7-ENT-26	Biology of gall midges affecting mangoes with special reference to the extent of damage	India	Yes	4-A-1
A7-ENT-28	Taxonomic studies of several families of Mallophaga (chewing lice)	India	No	
A7-ENT-29	A study of the taxonomy of adult and larval Bruchidae	India	Yes	20-A-5
A7-ENT-31	Investigations of insect pests of sorghum and millets	India	Yes	7-G-2
A7-ENT-33	Hereditary variations in the ability of Myzus persicae to transmit potato leafroll and virus "Y"	India	Yes	2-H-1
A7-ENT-35	Biology of gall midges affecting citrus plants with special reference to extent of damage	India	Yes	4-A-1
A7-ENT-37	Taxonomic survey of the hymenopterous parasites belonging to the family Ichneumonidae in India	India	No	

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Inc. in Summary of Progress	Line Proj. Inc. in Area & Sub-heading
A7-ENT-40	A study of factors affecting the dissemination of Coccinella septempunctata	India	Yes	21-C-17
A7-ENT-42	Survey for natural enemies of aphids in India	India	Yes	21-A-4
A7-ENT-44[1/]	Physiological factors governing susceptibility or resistance of crop plants to leafhoppers	India	Yes	1-G-2 1-H-2
A7-ENT-47[1/]	Biology of gall midges affecting fig fruits with special reference to the extent of damage and its relationship to the spread of disease organisms	India	Yes	4-A-1
A7-ENT-51[1/]	Studies on the systematics of the aphid genus Microspiphum (Homoptera- Aphidae)	India	No	
A10-ENT-5	Host plant-vector and host plant-virus relationships of the rough dwarf virus of corn and methods for control of the disease	Israel	Yes	7-H-1
A10-ENT-6	Acoustic responses of the desert locust (Schistocerca gregaria), Moroccan locust (Dociostaurus maroccanus) and (Acrotylus insurbricus) (Orthoptera; Acrididae)	Israel	Yes	5-A-1
A10-ENT-10[1/]	Biology of natural enemoes of citrus scale insects, in order to develop methods of their mass production for biological control	Israel	Yes	21-C-15
A10-ENT-12	Laboratory study of tick repellents and acaricides	Israel	No	
A10-ENT-13[1/]	Factors influencing variations in insecticide resistance	Israel	No	
A10-ENT-15[1/]	A study of the ecology, biology, and control of the citrus bud mite (Aceria sheldoni) Eriophyidae	Israel	No	
A13-ENT-3	Investigations on the biology of dung beetles in Korea and their role in the prevention of fly breeding in dung	Korea	No	
A17-ENT-5[2/]	Studies on indigenous natural enemies of scale insects and fruit flies	Pakistan	Yes	4-D-1
A17-ENT-7	Investigations on the natural enemies of corn borers	Pakistan	Yes	21-C-20
A17-ENT-8	Studies on the natural enemies of insect pests of rice	Pakistan	Yes	21-C-16
A17-ENT-9[2/]	Studies on the insect enemies of noxious weeds in Pakistan	Pakistan	No	
A17-ENT-10	Studies on oriental leafhoppers (Typhlocybinae)	Pakistan	Yes	20-A-7
A17-ENT-13	Insects, other plant-feeding organisms or plant diseases which attack Eurasian watermilfoll	Pakistan	Yes	21-B-3
A17-ENT-14[1/]	Biologies and host plant ranges of insects that attack noxious weeds common to Pakistan and the United States	Pakistan	No	
A17-ENT-15[1/]	Basic studies of parasites of the green peach aphid in northern West Pakistan	Pakistan	No	
A17-ENT-16[1/]	Relations between the paraslte-predator complex and the host plants of scale insects in Pakistan	Pakistan	No	
E8-ENT-1[2/]	An investigation of the population dynamics of Calligypona pellucida (F.) and of the nature of the injury to oats and spring wheat caused by this plant hopper	Finland	Yes	7-H-2
E11-ENT-1	Control of the olive fly, (Dacus oleae (Gmelin)) with radiation or chemical sterilization procedures	Greece	Yes	4-A-2

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
E15-ENT-1	Study of Acarine disease of honey bees	Italy	Yes	18-C-14
E21-ENT-2 2/	Studies on the possibility of biological control of aphids and scale insects and the effect of pesticides on the natural enemies of these pests	Poland	Yes	3-B-2
E21-ENT-3 2/	The influence of some vitamins on the physiology of the Colorado potato beetle (Leptinotarsa decemlineata Say)	Poland	No	
E21-ENT-4	The causes and the role of diapause of insect pests	Poland	No	
E21-ENT-5 2/	Studies on the differences in susceptibility of spider mites to acaricides and on cholinesterases in spider mites as influenced by acaricides	Poland	Yes	3-B-2
E21-ENT-6 2/	The nature of infectious processes caused by protozoa in insects	Poland	No	
E21-ENT-7 2/	The development, maturation and production of drones and natural mating of virgin and drone honey bees	Poland	Yes	18-A-5
E21-ENT-8	Mite fauna of orchards with special reference to the relation between phytophagous and predaceous species	Poland	Yes	3-D-4
E21-ENT-9	Insect vectors of virus diseases of various forage legumes	Poland	Yes	5-H-3
E21-ENT-10	Studies on distance of mating flights of honey bee queens and drones	Poland	Yes	18-A-6
E21-ENT-11 1/	Role of parasitic Hymenoptera in reduction of population size of two species of the genus Lygus	Poland	No	
E21-ENT-12	The role of nematodes as factor reducing populations of insect pests	Poland	No	
E21-ENT-14 1/	Studies regarding the bionomics, economic importance and natural control factors affecting Oulema species (cereal leaf beetle) in Poland	Poland	No	
E21-ENT-15 1/	Biology and utilization of diploid drone honey bees	Poland	Yes	18-A-5
E21-ENT-16 1/	The population trends in predaceous arthropods in apple orchards sprayed with different pesticides and the influence of these trends on the population density of phytophagous mites and some other pests	Poland	No	
E21-ENT-17 1/	Studies on interactions of various pathogens in one insect host (cutworms)	Poland	No	
E21-ENT-18 1/	Study on the influence of essential fatty acids and alpha-tocopherol on the lipid metabolism and physiology of Colorado potato beetle, Leptinotarsa decimlineata (Say), and on the vitamin activity of alpha-tocopherol, especially in coming generations	Poland	No	
E21-ENT-19 1/	Relationships in parallel development of insect host and parasite resistance to a common toxicant	Poland	No	
E30-ENT-1 1/	Bioecological investigations of the pink bollworm (Pectinophora - Platyedra gossypiella Saund.) under the conditions of the southeast of the SR Macedonia	Yugoslavia	No	
E30-ENT-2 1/	Investigations of leaf miners in orchards	Yugoslavia	No	
E30-ENT-3 1/	Parasites, predators, and pathogenic organisms study of the cereal leaf beetle, (Oulema melanopa L.), and resistance of domestic and small grain varieties to the insect	Yugoslavia	No	

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
F4-ENT-2	Survey of the insect fauna of Egypt	Egypt	Yes	20-A-9
F4-ENT-3 2/	Induced sterility in males of the Mediterranean fruit fly, Ceratitis capitata, as a means of controlling and possibly eradicating that pest	Egypt	No	
F4-ENT-4	Biology, ecology and utilization of insects other than honey bees in the pollination of agricultural crops	Egypt	Yes	18-F-3
F4-ENT-5 1/	Studies on the insects attacking thistles in the U.A.R.	Egypt	No	
F4-ENT-6 1/	Studies of the control of houseflies and mosquitoes by means of chemosterilants in Egypt	Egypt	No	
F4-ENT-8 1/2/	Purchase of synoptic collection of Egyptian insects (includes bees, wasps, beetles and moths)	Egypt	No	
S3-ENT-1	Biology and breeding of honey bees	Brazil	Yes	18-A-7
S3-ENT-7	Catalogue of insects living on plants in Brazil and of the parasites and predators of the insects	Brazil	Yes	21-A-2
S5-ENT-2	A biochemical study of Drosophila (vinegar flies) classification	Colombia	Yes	20-A-3
S5-ENT-3	The metabolism of temperature-acclimated Drosophila	Colombia	No	
S9-ENT-1 2/	Studies of the parasites and predators of several insects of economic importance	Uruguay	Yes	21-A-3
S9-ENT-3 2/	Investigations on the biology and biological control of the fire ant, Solenopsis saevissima richteri, in Uruguay	Uruguay	Yes	13-C-3 17-C-3
S9-ENT-6	Systematic collections, identification, and classification of the grasshoppers of Uruguay and neighboring territories of southern Brazil, southern Paraguay, and adjacent provinces of Argentina	Uruguay	Yes	20-A-2
S9-ENT-7 1/	Investigations on natural enemies of ants	Uruguay	No	

1/ Initiated during reporting year.
2/ Terminated during reporting year.

DOBBS BROS.
DEC. 1974


U.S. DEPARTMENT OF AGRICULTURE
NATIONAL AGRICULTURAL LIBRARY


UNITED STATES DEPARTMENT OF AGRICULTURE
NATIONAL AGRICULTURAL LIBRARY
U.S. DEPARTMENT OF AGRICULTURE